IJOEAR

International Journal of Environmental & Agriculture Research

www.ijoear.com

ISSN
2454-1850

Volume-4, Issue-2, February 2018

Preface

We would like to present, with great pleasure, the inaugural volume-4, Issue-2, February 2018, 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:

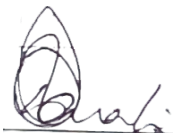
Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, 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.

Mukesh Arora
(Editor-in Chief)



Dr. Bhagawan Bharali
(Managing Editor)

Fields of Interests

Agricultural Sciences	
Soil Science	Plant Science
Animal Science	Agricultural Economics
Agricultural Chemistry	Basic biology concepts
Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
Crop Production	
Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth	Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed
Pulse Crops: Peas (all types), field beans, faba beans, lentils, soybeans, peanuts and chickpeas.	Hay and Silage (Forage crop) Production
Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.)	Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries)
Tree Nut crops: Hazlenuts. walnuts, almonds, cashews, pecans	Berry crops: strawberries, blueberries, raspberries
Sugar crops: sugarcane. sugar beets, sorghum	Potatoes varieties and production.
Livestock Production	
Animal husbandry	Ranch
Camel	Yak
Pigs	Sheep
Goats	Poultry
Bees	Dogs
Exotic species	Chicken Growth
Aquaculture	
Fish farm	Shrimp farm
Freshwater prawn farm	Integrated Multi-Trophic Aquaculture
Milk Production (Dairy)	
Dairy goat	Dairy cow
Dairy Sheep	Water Buffalo
Moose milk	Dairy product
Forest Products and Forest management	
Forestry/Silviculture	Agroforestry
Silvopasture	Christmas tree cultivation
Maple syrup	Forestry Growth
Mechanical	
General Farm Machinery	Tillage equipment
Harvesting equipment	Processing equipment
Hay & Silage/Forage equipment	Milking equipment
Hand tools & activities	Stock handling & control equipment
Agricultural buildings	Storage

Agricultural Input Products	
Crop Protection Chemicals	Feed supplements
Chemical based (inorganic) fertilizers	Organic fertilizers
Environmental Science	
Environmental science and regulation	Ecotoxicology
Environmental health issues	Atmosphere and climate
Terrestric ecosystems	Aquatic ecosystems
Energy and environment	Marine research
Biodiversity	Pharmaceuticals in the environment
Genetically modified organisms	Biotechnology
Risk assessment	Environment society
Theoretical production ecology	horticulture
Breeding	plant fertilization

Board Members

Dr. V K Joshi

Professor V.K.Joshi is M.Sc., Ph.D. (Microbiology) from Punjab Agricultural University, Ludhiana and Guru Nanak Dev University, Amritsar, respectively with more than 35 years experience in Fruit Fermentation Technology, Indigenous fermented foods, patulin ,biocolour ,Quality Control and Waste Utilization. Presently, heading the dept. of Food Science and Technology in University of Horticulture and Forestry, Nauni-Solan (HP), India.

Mr. Aklilu Bajigo Madalcho

Working at Jigjiga University, Ethiopia, as lecturer and researcher at the College of Dry land Agriculture, department of Natural Resources Management.

Dr. Vijay A. Patil

Working as Assistant Research Scientist in Main Rice Research Centre, Navsari Agricultural University, Navsari. Gujarat- 396 450 (India).

Dr. S. K. Jain

Presently working as Officer Incharge of All India Coordinated Sorghum Improvement Project, S. D. Agricultural University, Deesa, Gujarat.

Dr. Salvinder Singh

Presently working as Associate Professor in the Department of Agricultural Biotechnology in Assam Agricultural University, Jorhat, Assam.

Dr. Salvinder received MacKnight Foundation Fellowship for pre-doc training at WSU, USA – January 2000- March 2002 and DBT oversease Associateship for Post-Doc at WSU, USA – April, 2012 to October, 2012.

Mr. Anil Kumar

Working as Junior Research Officer/Asstt. Prof. in the dept. of Food Science & Technology in Agriculture & Technology, Pantnagar.

Table of Contents

Climate Risk Insurance for Resilience: A Systematic Review

Oladayo Nathaniel Awojobi

Department of Social Security, Bonn-Rhein-Sieg University of Applied Sciences, Sankt Augustin, Germany

Abstract**—** *This study developed and conducted a systematic mixed-methods grey literature methodology to characterise and identify climate risk insurance initiative in building resilience in developing countries. The study found that climate risk insurance can help developing countries build resilience against extreme weather events. However, there are barriers to the initiative. This is because of the issue of lack of climate data instruments. The collaboration between the public and private sectors is one way to overcome the challenges of implementing climate risk insurance. This systematic review methodology presents crucial insights on the state-of-the-art knowledge on climate risk insurance and resilience in developing countries.*

Keywords**—** ***Climate Change, Climate Risk Insurance, Developing countries, Systematic Review.***

I. INTRODUCTION

According to the Munich Re's NaCatService, climate change impacts have quadrupled since 1992 (Schaefer & Waters, 2016). Climate change, harsh conditions like weather-related disasters, as well as slow-onset change like rising sea levels, undermine sustainable development and resilience, hinder socio-economic development and fortify cycles of poverty across the world(GIZ & BMZ, 2015).

Going by the 5th Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), the risks associated with harsh weather conditions will further increase with rising temperatures (Schaefer & Waters, 2016). This will lead to increasing poverty, especially in developing countries where they have a weak adaptive capacity and most of their citizens are engaged in commercial and subsistence farming for their livelihoods(Barrett et al., 2007).

The poorest in the world, especially the women bear a disproportionate burden of climate stress, despite the fact that they contribute less to the harming of the environment(Schaefer & Waters, 2016). Harsh weather conditions are reinforcing poverty cycles and the immediate-future predictions affirm that climate change will extremely increase the number of poverty-stricken people in developing countries (Hallegatte et al., 2016). As a result, there is growing need to support the most vulnerable people and poor countries in establishing effective strategies to manage risks and unexpected shocks and build resilience to climate change impacts (Schaefer & Waters, 2016). One feasible strategy to support the poor is through the climate risk insurance initiative (Lashley, 2012). Climate risk insurance is an instrument that provides security against loss of assets, livelihoods and lives arising from harsh weather conditions by providing effective and expeditious post-disaster relief on individuals, communities, national and regional levels(Schaefer & Waters, 2016).

To understand the connection between climate risk insurance and resilience, four key questions were asked: 1) Can climate risk insurance help developing countries reduce climate risk today? 2) What are the multiple factors driving climate risk insurance initiative? 3) What are the impacts?4) What constitute barriers to climate risk insurance? To answer these research questions, a systematic review of grey literature was undertaken. Certainly new in climate change research, systematic reviews present encouraging method to identify, analyse and synthesise a huge volume of literature (Porter et al., 2014). Contrary to conventional literature reviews, where search or selection criteria are often ambiguous and hardly favored, systematic reviews are more clear, understandable and reproducible (Porter et al., 2014).

The aim of this paper is to frame major issues and summarize the current state of knowledge about climate risk insurance as it relates to helping the poor to manage risks and unexpected shocks and build resilience to climate change impacts. The remainder of this paper is structured as follows: Section 2 demonstrates the methodology employs. Section 3 presents the results. Section 4 discusses the results and section 5 presents the conclusions.

II. MATERIALS AND METHODS

2.1. Systematic reviews

This paper adopts a systematic review methodology to examine climate risk insurance in building resilience in developing countries vis a vis helping the poor to adapt and cope against immediate and future climate shocks. A systematic review is an analysis and assessment of the state of knowledge on given research question, designed to rigorously summarize existing understanding (Ford, Berrang-Ford, & Paterson, 2011). Systematic reviews differ from conventional literature reviews in

three major ways(Ford & Pearce, 2010).First, they embody reviewing documents according to vividly developed questions (Ford & Pearce, 2010). Second, they embody full reporting of search and criteria for inclusion and exclusion of research articles separated in the analysis along with those included (Ford & Pearce, 2010). Finally, systematic selection of publishing materials allows the use of mixed-methods review of trends in the literature (Ford & Pearce, 2010).

2.2. Literature search and review

The literature search for the review embodies selection, coding and content analysis of the literature source. The literature search was done via an electronic search for peer-reviewed journals and grey literature in the disciplines of climate risk insurance, poverty and resilience. However, only grey literature was added to the selection criteria because most of the adaptation projects in developing countries are undertaken by international organizations that provide funds and evaluation research on the impact of climate risk insurance in helping the poor to cope and adapt to immediate and future climate shocks. And most of their climate publications are published on their websites as reports, policy briefs and documents.

The grey literature had to meet certain inclusion and exclusion criteria for this review. The inclusion basic terms were *"climate risk insurance"* and *"resilience""poverty."* The exclusion criterion was the narrowing the outcomes of the search to developing countries and excluding developed countries because developing countries have poor adaptive responses to climate change and climate change affects mostly the agricultural sector that employs a lot of people.

In all, 9 reviewed documents met the criteria of the study and an identifier number (#1-9) was assigned to each of the selected documents and is used to assign to each one individually. The summary of the literature reviewed is listed in Table 1. The 9 reviewed documents composed of 6 qualitative and 3 mixed-method approaches. Their thematic focus areas include climate change, vulnerability and climate risk insurance.

The selected documents were systematically analysed to ascertain: 1) if climate risk insurance can help developing countries reduce climate risk today; 2) what factors are driving climate risk insurance initiative; 3) if there areimpacts;4) whether they are barriers to the implementation. The selected documents that met the criteria were analysed using content analysis technique.

TABLE 1
SUMMARY SYSTEMATIC REVIEWED ARTICLES ON CLIMATE RISK INSURANCE ON RESILIENCE

Identifier	Study	Type of study	Method	Thematic focus	Data Source
1	GIZ (2017)	Factsheet	-	Climate risk insurance, climate risk management	Grey literature
2	GIZ and GMZ (2015)	Background paper	Mixed	Climate change, climate- insurance	Grey literature
3	Hermann et al. (2016)	Working paper	Qualitative	Climate risk insurance	Grey literature
4	Ogden et al. (2015)	Analysis	Qualitative	Climate risk insurance	Grey literature
5	RESULTS UK (2016)	Policy	Qualitative	Climate risk insurance, pro- poor principles	Grey literature
6	Schaefer & Waters (2016)	Research paper	Mixed	Climate risk insurance, vulnerability, impact studies	Others
7	Warner et al. (2009)	Analysis	Qualitative	Adaptation, climate change, disaster risk reduction, insurance	Grey literature
8	Warner et al. (2013)	Working paper	Mixed	Climate risk insurance, climate risk management	Grey literature
9	Yuzva et al. (2014)	Project	Qualitative	Climate risk insurance, climate risk management	Grey literature

III. RESULTS

3.1 Can climate risk insurance help developing countries reduce climate risk today?

Climate risk insurance was explicitly expressed (Table 2) in 9 (100%) of the publications that were reviewed that it can help the poor and poor developing countries in building resilience due to climate change *vis à vis* risks associated with extreme weather events. For instance, climate risk insurance can help the poor, vulnerable communities and poor countries in

providing security against loss of lives and means of livelihoods in the aftermath of disaster period; establishing reliable and dignified aftermath disaster relief, setting incentives for prevention; providing certainty for climate-affected public and private investments, and mitigating disaster-related poverty and stimulating economic development (#2).

TABLE 2
CAN CLIMATE RISK INSURANCE HELP DEVELOPING COUNTRIES REDUCE CLIMATE RISK TODAY?

Source	Reviewed articles supporting the climate risk insurance and resilience hypothesis
GIZ (2017)	It provides protection against risks arising from extreme weather events
GIZ and GMZ (2015)	It can provide security against the loss of assets, livelihoods and lives in the post-disaster period
Hermann et al. (2016)	It might play a substantial role in protecting vulnerable countries from climate change
Ogden et al. (2015)	It can play a key role in reducing the risks of climate change in developing countries
RESULTS UK (2016)	It can also contribute to resilience building or adaptation
Schaefer & Waters (2016)	It can play a role in increasing the resilience of the poor and vulnerable people
Warner et al. (2009)	Could help smooth household incomes when shocks occur
Warner et al. (2013)	It can reduce immediate and long-term financial impact associated with extreme weather events
Yuzva et al. (2014)	It can reduce immediate and long-term repercussions from extreme weather events

3.2 What are the multiple factors driving climate risk insurance initiative?

Climate change is reported in all the 9 reviewed articles as the primary motivating factor for climate risk insurance. However, there are secondary motivating factors. For instance, six of the reviewed articles (#1, 2, 3, 5, 6, 7) think of *poverty* associated with climate change. Also, all reviewed articles want appropriate authorities to use climate risk insurance to support the most *vulnerable.* Six other articled (#1, 3, 5, 6, 7, 8) mentioned the need to help the *poor* when extreme climate events occur. Two reviewed articles (#3, 6) assert that extreme weather events will impair *socioeconomic development.* For this reason, (#2) claims that climate risk insurance should be introduced to spur *economic development* after math of weather-related disasters.

3.3 What are the impacts?

Seven out of the nine reviewed articles gave various forms of climate risk insurance impacting their beneficiaries. Table 3 lists the various impacts emanating from the systematically reviewed articles.

TABLE 3
WHAT ARE THE IMPACTS OF CLIMATE RISK INSURANCE?

Source	Evidence of climate risk insurance impacting their beneficiaries
Hermann et al. (2016:9)	"The initiative provides insurance, among others, to poor farmers and other food insecure households, who are given access to insurance by paying into Insurance-for-Assets (IfA) with their own labour. When a drought hits, as indicated by a specified weather index, policyholders receive compensation"
Ogden et al. (2015)	Insurance was quickly expanded from 200 households in one village in 2009 to 13,000 households in 43 villages in 2011, yielding significant benefits
RESULTS UK (2016:2)	"Since I started taking insurance, I now have peace of mind and feel more confident when investing in my farm"
Schaefer & Waters (2016:64)	"Although insurance does not cover all the losses that farmers face when the rains fail, it still limits the financial losses and helps them not to resort to negative coping strategies" (Belay, Sophia. Email Interview. 15 April 2016).
Warner et al. (2009)	It enables participating farmers to increase their farm productivity
Warner et al. (2013)	It leads to significantly larger agricultural investment as well as to risk production choices to farmers
Yuzva et al. (2014:14)	"A market study in India found that rice farmers offered formal index insurance in the Andhra Pradesh region of India diversified their risks by planting higher-yield varieties of rice than those without index insurance"

3.4 What are the barriers to climate risk insurance?

The barriers to climate risk insurance initiative are presented in the majority of the articles reviewed here. Climate data challenges are widely noted, while it is considered that most developing countries lack adequate data collection instruments, there is also the issue of accurate climate data collection. Six out of the nine reviewed articles (#1, 4, 5, 6, 7. 9) mentioned climate data issues as barriers to implementing a climate risk insurance initiative in developing countries. For instance, (#1) claims "historical and up-to-date weather data is a precondition for climate risk insurances. However, data availability is still inadequate in many countries around the world" (GIZ, 2017, p. 2). On the other hand, two other reviewed articles (#8, 9) consider high start-up costs as a barrier to climate risk insurance in poor communities.

IV. DISCUSSION

The systematic review of this study shows that climate risk insurance has gained prominence more in grey literature than peer-reviewed literature. This can be attributed to the dominance of climate risk insurance for resilience by development organizations. In all the grey literature that was reviewed on climate risk insurance on resilience in developing countries, four major findings emanated.

Climate risk insurance is considered as a prerequisite for immediate and future impacts of climate change, especially in developing countries where most of the population that are vulnerable to climate change are poor. The poor adaptive capacity of poor developing countries when extreme weather events occur put poor people and communities in precarious situations. The climate risk insurance initiative from the findings of this study indicate that it can allow individuals, communities and poor countries build resilience when extreme weather events occur. However, the initiative not been embraced popularly in most developing countries from the reviewed articles. This can be owing to the notion that insurance is an investment that is usually undertaken by private investors, unlike cash transfers that are introduced by the governments in developing countries to assist the poor. Building public-private partnership approaches in conjunction with international support are exceptionally important for poor developing countries where pure market-based solutions are generally not achievable due to high start-up costs, lack of data and limited access or low demand for standard insurance products from the poor part of the society (Warner et al., 2013).

The impacts of climate change on poor communities put the inhabitants of these vulnerable communities in precarious conditions because of their inability to cope or adapt when extreme weather events occur. The Munich Climate Insurance Initiative (MCII) was initiated to address the poor situations of vulnerable communities because there are in disadvantaged position, as commended in the UN Framework Convention on Climate Change (MCII, 2017). Findings from the study revealed that climate change, the vulnerable position of the poor and the impacts of climate change on local economies have been identified as the major drivers for climate risk insurance. It helps to provide prompt and stable finance to cover parts of the economic losses, especially compared to ad hoc post-disaster financing alternatives such as aid, loans and family assistance(GIZ, 2017). This accedes for accelerated emergency assistance and reconstruction, helping to save lives, safeguard livelihoods and assets and protect development again (GIZ, 2017).

While some developing countries have implemented climate risk insurance with the support of stakeholders, evidence has shown that some of the beneficiaries have been impacted by the initiative. Findings from this study revealed that most of the beneficiaries are farmers who need to protect their farms as well as their means of livelihoods against extreme weather events. Various forms of climate risk insurance are being operated in many developing countries. The Climate Adaptation Development Program (CADP) was established in 2007 by Swizz Re in order to safeguard village clusters in Ethiopia, Kenya and Mail against extreme drought(UNFCCC, 2008). Similarly, in India, considerable numbers of disaster micro insurance schemes are in place, covering the loss of life or assets, among others, caused by natural disasters (Hermann et al., 2016). Well-packaged insurance products assist to decrease the economic impact of and expedite quick recovery after natural disasters (Warner et al., 2013). In consonance with insurance theory, insurance works by restoring "uncertain prospect of losses with a certainty of making small, regular payments (Warner et al., 2013). In turn, it can help safeguard livelihoods and risk prevention measures, risk transfer can help to strengthen resilience to climate change (Warner et al., 2013).

Finally, the study findings showed that barriers to climate risk insurance in developing countries were risk specific. For instance, inadequate climate data is considered as a major barrier to the implementation of climate risk insurance in developing counties coupled with the high start-up costs.

V. CONCLUSIONS

Systematic review formed the methodology of this study which examines the current state of knowledge of climate risk insurance in building resilience in developing countries. The study focused on grey literature for data collection because most studies on climate risk insurance were reports, documents and projects of organizations that are stakeholders in the climate risk insurance initiative.

The findings from the systematic reviewed showed that climate risk insurance can help individuals, communities and poor countries build resilience against extreme weather events. In addition, climate risk insurance is motivated by the impacts of climate change and evidence from this study has shown that some farmers have benefited from the initiative. Despite the importance of climate risk insurance, there are still barriers to the imitative in some developing countries.

If developing countries need to embrace the climate risk insurance initiative, the governments should be fully involved because no one is immune when extreme weather events occur. Building public-private partnership will go a long way in making climate risk insurance a successful apparatus for resilience in developing countries.

REFERENCES

[1] Barrett, C. B., Barnett, B. J., Carter, M. R., Chantarat, S., Hansen, J. W., Mude, A. G., … Ward, M. N. (2007). Poverty traps and climate risk: limitations and opportunities of index-based risk financing. *The International Research Institute for Climate Change and Society*.

[2] Ford, J. D., Berrang-Ford, L., & Paterson, J. (2011). A systematic review of observed climate change adaptation in developed nations: A letter. *Climatic Change*, *106*(2), 327–336. https://doi.org/10.1007/s10584-011-0045-5

[3] Ford, J. D., & Pearce, T. (2010). What we know, do not know, and need to know about climate change vulnerability in the western Canadian Arctic: a systematic literature review. *Environmental Research Letters*, *5*(1), 014008. https://doi.org/10.1088/1748-9326/5/1/014008

[4] GIZ. (2017). Understanding climate risk insurance. Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ). Retrieved from http://www.insuresilience.org/wp-content/uploads/2017/11/171009_giz_insuresilience_factsheet_defining-climate-risk_rz_01_ansicht.pdf

[5] GIZ, & BMZ. (2015). CLIMATE RISK INSURANCE FOR STRENGTHENING CLIMATE RESILIENCE OF POOR PEOPLE IN VULNERABLE COUNTRIES: A BACKGROUND PAPER ON CHALLENGES, AMBITIONS AND PERSPECTIVES. Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH. Retrieved from http://www.climate-insurance.org/fileadmin/mcii/pdf/G7_Climate-Risk-Insurance_Background-Paper.pdf

[6] Hallegatte, S., Bangalore, M., Bonzanigo, L., Fay, M., Narloch, U., Rozenberg, J., & Vogt-Schilb, A. (2014). Climate change and poverty–an analytical framework. *World Bank*. Retrieved from https://openknowledge.worldbank.org/bitstream/handle/10986/20639/WPS7126.pdf?sequence=1&isAllowed=y

[7] Hermann, A., Köferl, P., & Mairhöfer, J. P. (2016). Climate Risk Insurance: New Approaches and Schemes. Retrieved from https://www.allianz.com/v_1479816006000/media/economic_research/publications/working_papers/en/ClimateRisk.pdf

[8] Lashley, J. (2012). Climate Risk Insurance: A tool for climate risk management and poverty reduction. Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ). Retrieved from http://www.climate-insurance.org/fileadmin/mcii/documents/201304_MCII_Carib_ClimateRiskInsurance_factsheet.pdf

[9] MCII. (2017). Hamburg: G20 Countries should address resilience to warming planet. Retrieved January 23, 2018, from http://www.climate-insurance.org/fileadmin/mcii/documents/20170707_G20_press_statement_MCII_final.pdf

[10] Ogden, P., Bovarnick, B., & Hoshijima, Y. (2015). Key Principles for ClimateRelated Risk Insurance. Center of American Progress. Retrieved from https://cdn.americanprogress.org/wp-content/uploads/2015/08/26131302/ClimateRiskInsurance-report.pdf

[11] Porter, J. J., Dessai, S., & Tompkins, E. L. (2014). What do we know about UK household adaptation to climate change? A systematic review. Sustainability Research Institute (SRI), School of Earth and Environment, The University of Leeds, Leeds, LS2 9JT, United Kingdom.

[12] RESULTS UK. (2016). Ensuring Climate Risk Insurance Works for the Poor. Retrieved from http://www.results.org.uk/sites/default/files/files/Ensuring%20Climate%20Risk%20Insurance%20Works%20for%20the%20Poor.pdf

[13] Schaefer, L., & Waters, E. (2016). CLIMATE RISK INSURANCEFOR THE POOR & VULNERABLE: HOW TO EFFECTIVELY IMPLEMENT THE PRO-POOR FOCUS OF INSURESILIENCE. MUNICH CLIMATE INSURANCE INITIATIVE.

[14] UNFCCC. (2008). Mechanisms to manage financial risks from direct impacts of climate change in developing countries: Technical paper. Retrieved January 23, 2018, from http://unfccc.int/resource/docs/2008/tp/09.pdf

[15] Warner, K., Ranger, N., Surminski, S., Arnold, M., Linnerooth-Bayer, J., Michel-Kerjan, E., … Herweijer, C. (2009). *Adaptation to climate change: Linking disaster risk reduction and insurance*. United Nations International Strategy for Disaster Reduction Geneva.

[16] Warner, K., Yuzva, K., Zissener, M., Gille, S., Voss, J., & Wanczeck, S. (2013). *Innovative Insurance Solutions for Climate Change: How to integrate climate risk insurance into a comprehensive climate risk management approach*. UNU-EHS.

[17] Yuzva, K., Zissener, M., & Warner, K. (2014). Countries addressing climate change using innovative insurance solutions. United Nations University Institute for Environment and Human Security (UNU-EHS).

Kinetics and Mathematical Modeling of Microwave Drying of Sri Lankan Black Pepper (Piper nigrum)

B.M.W.P.K. Amarasinghe[1*], A.J.M.L.M. Aberathna[2], K.K.P.P. Aberathna[3]

Department of Chemical and Process Engineering, University of Moratuwa, Sri Lanka

Abstract— *Drying characteristics of black pepper (Piper nigrum) was investigated in a microwave drying system. Drying experiments were carried out at three different microwave power levels, 180, 360 and 540 W and the moisture content was measured at different time intervals. Experimental results were fitted to seven thin layer drying models; Newton, Page, Henderson and Pabis, Logarithmic, Midilli et. al., Weibull and Kaleta et. al.. Statistical indicators; Coefficient of determination (R^2), Root mean square error (RMSE) and reduced chi square values (χ^2) showed Midilli et. al., Weibull and Kaleta et. al. models give better fit to the experimental values. Drying rate constants and the equation constants were compared and analyzed. Similarities of the drying models were observed and discussed with respect to the equation parameters. The absence of the constant drying rate period in drying curves show the drying of black pepper lies totally in the falling rate period where the drying rate is controlled by the moisture diffusion. Maximum drying rates observed were 0.02, 0.05 and 0.08 kg moisture/kg of dry material/ min at 180, 360 and 540 W power levels. Results revealed drying rate constant and the effective moisture diffusivity values increases with the microwave power level. Drying rate constants were 0.03, 0.09 and 0.16 min^{-1} and the effective moisture diffusivity values were 2.43 x 10^{-10}, 4.87 x 10^{-10}, 1.42 x 10^{-9} m^2/s for power levels of 180, 360 and 540 W respectively. The Activation energy of black pepper calculated based on the Arrhenius equation is 86.7 W/g.*

Keywords— Black pepper, Microwave drying, thin layer drying models, diffusivity.

I. INTRODUCTION

Black pepper (BP) (*Piper nigrum*) is the most widely used spice in the world. BP is also used in perfume and pharmaceutical industries. The crop is grown in many tropical countries including Sri Lanka. The world production of pepper in 2017 is approximately 472,500 MT and is gradually growing. Sri Lanka produces around 3% of the world pepper production [1].

The mature peppercorns are green in colour and become red when ripe (Fig 1). This mixture of green and red peppercorns is dried after harvesting to reduce the moisture content to avoid fungal growth and wastage. The moisture content of the pepper is around 77% when harvested and reduced up to 11% (wet basis) before storage. Solar drying is the widely used method in Sri Lanka for drying of black pepper. However, improper drying specially during rainy season causes postharvest losses. National Committee on Postharvest Technology & Value Addition in Sri Lanka has identified BP as a high priority crop and further development of postharvest drying technologies is recommended as a main thrust research area [2].

Food and grain drying have been paid much attention by researchers. Solar drying, hot air drying, fluidized bed or spouted bed drying are the widely used techniques for food drying [3-8]. Application of Microwave technology has been investigated over the last few years for food and grain drying [9-13]. However, despite the wide use of BP as a spice in the world, the previous studies on drying of black pepper is limited. Solar tunnel dryers and fluidized bed or spouted bed dryers have been tested for black pepper drying [14-16].

Chacko *et. al.* [16] and Magda *et. al*. [13] have found that microwave drying is suitable for BP drying and have observed improvement in aroma despite minor losses of some volatiles. Drying kinetics is important in determining the drying time required and hence the dryer size.

Therefore, the objective of this work is to study drying kinetics of black pepper in microwave drying and determine moisture diffusivity and activation energy of the material.

FIG. 1: BLACK PEPPER PLANT (LEFT) AND DRY SEEDS (RIGHT)
(http://world-crops.com/black-pepper/ and http://www.fruitsvegetablecinnamonsuppliersrilanka.com)

II. THEORY

Drying is an important unit operation which involves simultaneous heat and mass transfer. The knowledge on drying kinetics, which determines the drying time and hence dryer size, is essential in dryer design. Numerous investigations have been reported on thin layer drying models. Newton or Lewis, Page, Henderson and Pabis, Logarithmic, Midilli *et.al* are among the widely used thin layer drying models to express drying kinetics. Some researchers have used less common models such as Weibull model. Kaleta *et.al.* have introduced several new models in their studies [3,5,17,18].

The moisture content (MC) of a material is expressed either on dry basis or wet basis. The dry basis moisture content is given by the equation (1).

$$MC = \frac{W - W_d}{W_d} \quad [1]$$

Where W and W_d are the weight of the material and weight of the dry material respectively

Equilibrium moisture content is negligible compared to the moisture content and the moisture ratio (MR) can be determined by equation (2).

$$MR = \frac{MC_t}{MC_i} \quad [2]$$

Where MC_i, MC_t are the Initial Moisture content and Moisture content at time t respectively.

The rate of drying largely depends on the rate of moisture diffusion within the solid material. Applying Fick's second law of diffusion for spherical particles leads to equation (3) which relates moisture ratio to the time of drying [8,19].

$$MR = \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2} exp\left[\frac{-n^2\pi^2}{R}\left(\frac{D_{eff}\, t}{R}\right)\right] \quad [3]$$

The equation (3) can be simplified in the following form;

$$ln(MR) = ln\left(\frac{6}{\pi^2}\right) - \left(\frac{\pi^2 D_{eff}}{R^2}\right) t \quad [4]$$

Where

n – number of terms
R- Particle radius (m)
D_{eff} - effective moisture diffusivity (m^2/s)
t – time (s)

The effective moisture diffusivity (D_{eff}) in solids is a function of the temperature and the moisture content. The temperature dependence of the diffusivity is adequately described by the Arrhenius equation as given by equation (5)[8].

$$D_{eff} = D_0 \, exp\left(\frac{-E_a}{RT}\right) \quad [5]$$

Where D_0 is the pre exponential factor (m^2/s) and T is the absolute temperature (K), R is the universal gas constant (J mol^{-1} K^{-1}), E_a is the Activation energy (J/mol).

For microwave drying, temperature measurement inside the dryer is not very precise and hence the modified versions of the Arrhenius equation, given by equations (6) and (7) are used to determine the activation energy [8,20].

$$k = k_o \; exp\left(-\frac{E_a m}{P}\right) \quad [6]$$

$$D_{eff} = D_0 \, exp\left(\frac{-E_a m}{P}\right) \quad [7]$$

k- drying rate constant (min^{-1})
k_0- pre exponential factor (min^{-1})
D_0- pre exponential factor (m^2/s)
E_a - Activation energy (W/g)
m –mass of raw sample (g)
P – microwave power (W)

III. METHODOLOGY

Matured fresh black peppercorns just harvested were provided by the local farmers. Microwave oven (singer, Model no.SMW23GA9, 23 L capacity) of variable power output setting and rated capacity of 900W was used for drying. The moisture content of pepper was determined by the oven drying method using the electric oven (model: LDO-060 E-DaihanLabTech Co Ltd).

20 g of black pepper was placed in the container as a thin layer and the microwave oven was set to the required power level. The weight of the sample was determined at selected time intervals using the analytical balance. The samples were weighed fast to avoid any interference with the drying process. The drying experiments were repeated for 3 power levels (180W, 360 W and 540 W). The pepper particle size was determined by the gravity settling method. Average size of the pepper particles were 4 mm diameter. All the experiments were duplicated to confirm the repeatability.

The moisture content (MC) of the pepper was calculated as a function of the time (equation (1)) and converted to moisture ratio (MR) values (equation (2)) for three different microwave power levels.

The MR as a function of time results were fitted to the seven different thin layer drying models given in Table 1 using Excel Solver. Among the large number of thin layer drying models the seven models were carefully selected. Kucuk *et. al*. have summarized and analyzed 67 thin layer drying models developed and used by various researchers. Simple and most widely used models such as Newton, Page and less common models namely Weibull, Kaleta *et. al*.were selected for data fitting so that the models can be compared [3].

TABLE 1
THIN LAYER DRYING MODELS[3,5,8]

Model Name	Model Equation	Comments
Newton or Lewis	$MR = exp(-kt)$	Simplest model, one model constant
Page	$MR = exp(-kt^n)$	Modification of the Newton's model with exponent 'n'. Commonly used model. Reported as the second best model for most products.
Henderson and Pabis	$MR = a \, exp(-kt)$	Modification of the Newton's model with constant 'a'. Ninth-best model
Logarithmic	$MR = a \, exp(-kt) + b$	Third best model. Second term 'b' included.
Midilli *et al*	$MR = a \, exp(-kt^n) + bt$	Reported as the best. However, 4 model constants.
Weibull	$MR = a \, exp(-kt^n) + b$	One form of the common Weibull equation. Twelfth best model.
Kaleta *et al*	$MR = a \, exp(-kt^n)$	Modification to the Page model with the parameter 'a'.

k in the equations in the Table 1 are drying rate constants and *a*, *b*, and *n* are the equation constants.

Three statistical parameters; Coefficient of determination (R^2), Root mean squar eerror (RMSE) and reduced chi square values (χ^2) given by equations 8,9 and 10 respectively, were used to determine the best fitted model [3,17,10].

$$R^2 = \frac{\sum_1^n (MR_{pre,i} - MR_{avg})^2}{\sum_1^n (MR_{exp,i} - MR_{avg})^2} \quad [8]$$

$$\chi^2 = \frac{\sum_1^n (MR_{exp,i} - MR_{pre,i})^2}{N - z} \quad [9]$$

$$RMSE = \left[\frac{1}{N}\sum_1^n (MR_{pre,i} - MR_{exp,i})^2\right]^{1/2} \quad [10]$$

Where

MR_{exp}, MR_{pre} and MR_{avg} are the experimental, predicted and average moisture ratios.

N – Number of observations z – Number of constants

Rate of drying (N), kg of moisture evaporated/kg of dry material/ min, was calculated using the equation 11, where *d(MC)/dt* is the gradient of moisture content (MC) as a function the time plot.

$$N = \frac{d(MC)}{dt} \quad [11]$$

IV. RESULTS AND DISCUSSION

The experimentally determined moisture content values were converted to the moisture ratio values and the data were fitted to the seven different thin layer drying models given in Table 1. The model parameters were determined using Excel Solver and the results are summarized in theTable2. Results show that all the seven models under investigation give a good fit to an acceptable level. However, statistical parameters shows that the Midilli *et. al*, Weibull and Kaleta *et. al* models give better fit to the experimental values. Experimental results and model predicted results using the Midilli *et.al*. equation are shown in Fig. 2.

Drying rate constant, k, values for all the models are in the range 0.02-0.03 $min^{-1}$for 180 W power levels, 0.07-0.09 $min^{-1}$for 360 W power level and 0.14-0.19 $min^{-1}$for 540 W power level. Values of the constant, *a*, in the Weibull, Kaleta *et. al*., Henderson and Pabis, Logarithmic and Midilli *et.al*. equations are approximately equal to one (1). This can be compared with the Lewis model and the Page model equations where *'a'* term is not appearing, which implies that the constant *'a'* is equal to one. Constant *'n'* in the Weibull, Kaleta *et. al*, Page and Midilli *et. al* equations are also approximately equal to one which are comparable with the Lewis, Hendarson & Pabis and Logarithmic models where the exponent of *'t'* is equal to one. Further, the very low parameter *'b'* values show that the effect of the second term in the Weibull, Logarithmic and Midilli *et. al.* equations are less significant. The above comparison of the parameters shows the similarity of the seven model equations valid for drying of black pepper in microwave drying.

The rate of drying can be determined using the gradient of moisture content (MC) vs time plot and the Fig. 3 shows drying rate (N) as a function of moisture content at three different microwave power levels. Constant drying rate period is negligible or not in existence showing that drying of BP in microwave is completely under the falling rate period. This shows diffusion of moisture within the solid is the rate controlling step in BP drying. This result is in agreement with the previous workers results on drying of food materials where the constant drying period is negligible [17].

Chacko *et. al.*[16] and Magda *et. al.* [13] showed that microwave oven is suitable for pepper drying; however low power range in suitable to avoid loss of volatiles. Therefore, 180 W powers are recommended for drying of BP in a microwave.

TABLE 2
THIN LAYER DRYING MODELS AND PARAMETERS FOR BLACK PEPPER DRYING IN A MICROWAVE OVEN (k in min^{-1})

Model	P (W)	Model Parameters	RMSE	R^2	χ^2
Newton or Lewis $MR = exp(-kt)$	180	k =0.0356	0.0185	0.9960	0.000347
	360	k =0.0726	0.0175	0.9957	0.000313
	540	k =0.1882	0.0242	0.9935	0.000620
Page $MR = exp(-kt^n)$	180	k =0.0268, n =1.0827	0.0132	0.9980	0.000179
	360	k = 0.0792, n =0.9687	0.0167	0.9961	0.000293
	540	k =0.1459, n =1.1369	0.0125	0.9983	0.000175
Henderson and Pabis $MR = a\ exp(-kt)$	180	*a* = 1.0459, k =0.0374	0.0105	0.9987	0.000112
	360	*a*=1.0018, k=0.0727	0.0174	0.9957	0.000313
	540	*a*=1.0432, k=0.1960	0.0197	0.9956	0.000442
Logarithmic $MR = a\ exp(-kt) + b$	180	*a*=1.0449, k=0.0376,b=0.0017	0.0105	0.9987	0.000113
	360	*a*=0.9960, k =0.0758,b=0.0133	0.0169	0.9959	0.000307
	540	*a* =1.0757, k =0.1713,b=0.0514	0.0119	0.9984	0.000171
Midilli *et al* $MR = a\ exp(-kt^n) + bt$	180	*a*=1.0317, k =0.0312 n =1.0536,b =0.000143	0.0094	0.9989	9.3012E-05
	360	*a* = 1.0368, k = 0.0955, n=0.9096, b=-0.0002	0.0153	0.9967	0.000256
	540	*a* = 1.0129,k =0.1603 n=1.0716, b=0.0014	0.0105	0.9987	0.000141
Weibull $MR = a\ exp(-kt^n) + b$,	180	*a*=1.0095, k =0.0302 n=1.0709b=0.0198	0.0090	0.9991	8.6998E-05
	360	*a*=1.049,k =0.0950 n=0.9061,b =0.0128	0.0153	0.9967	0.000262
	540	*a*=1.0409, k =0.1576 n=1.0664,b = 0.0279	0.0107	0.9987	0.000147
Kaleta *et al* $MR = a\ exp(-kt^n)$	180	*a* =1.0363,k=0.0338 n=1.0270	0.0100	0.9988	0.000104
	360	*a* = 1.0328,k=0.0912 n= 0.9298	0.0155	0.9966	0.000257
	540	*a*=1.0074,k=0.1500 n=1.1262	0.0123	0.9983	0.000182

4.1 Moisture diffusivity

Rate of drying hence the time required for drying depends on the moisture diffusivity within the solid. Equation (4) can be used to determine the moisture diffusivity within the solid. Plots of Ln(MR) as a function of time are shown in Fig.4. Average Effective moisture diffusivity values were calculated using the gradient of the plot and the results are shown in the Table 3. The diffusivity values obtained are in the same order of magnitude as the values obtained by other researchers for similar materials such as orange seeds and dika (Irvingiagabonensis) nuts and kernels [4,21]. Activation energy of pepper can be calculated using the Arrhenius type equation (5). Temperature measurements are only approximate in microwave ovens and therefore equations (6) and (7) which relates the drying rate constant (*k*) to the ratio of dry solid weight to the microwave power (*m/P*) is recommended for microwave drying. Fig. 5 shows a plot the drying rate constant values calculated using Midilli *et. al.* equation as a function of the ratio of dry solid weight to the microwave power (m/P). The

activation energy of the pepper thus calculated is 89.4 W/g and the k_o value is 0.3484 min^{-1}. Similarly, effective diffusivity values calculated can be used to determine the activation energy of the BP using equation (7) and the activation energy calculated is 86.7 W/g and the pre-exponential term, D_0, was 2.0 x 10^{-9} m^2/s.

Therefore, based on the above results equations (11) and (12) are proposed to determine the drying rate constant and the effective moisture diffusivity for BP drying in a microwave oven.

$$k = 0.3484\ exp\left(-\frac{89.4\ m}{P}\right) \quad [11]$$

$$D_{eff} = 2.0 \times 10^{-9}\ exp\left(\frac{-86.7\ m}{P}\right) \quad [12]$$

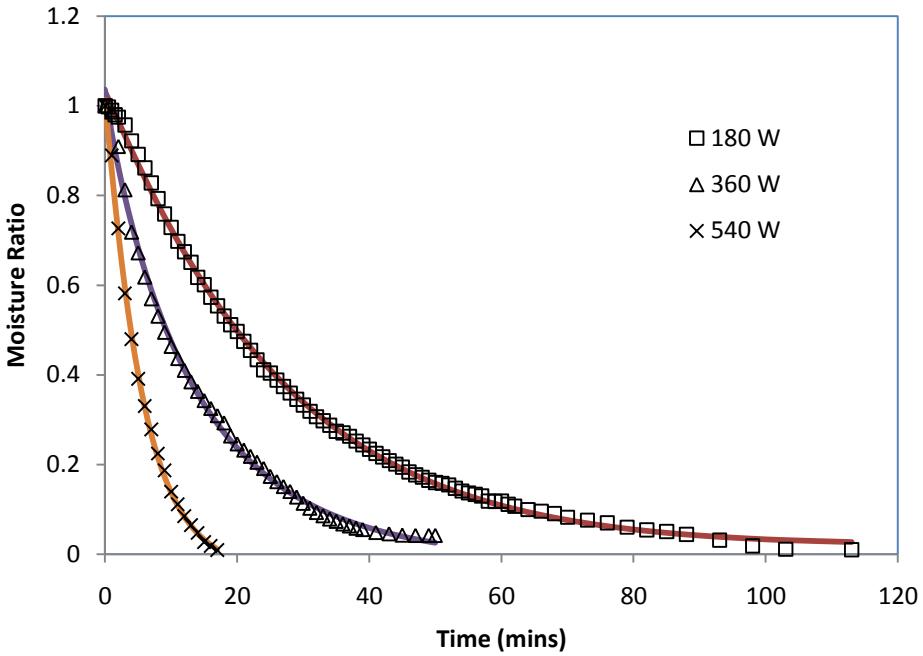


FIG 2: VARIATION OF MOISTURE RATIO (MR) OF BLACK PEPPER AS A FUNCTION OF TIME FOR THREE MICROWAVE POWER LEVELS (□, Δ, ×-EXP VALUES, SOLID LINES - PREDICTED CURVES MIDILLI ET. AL. MODEL)

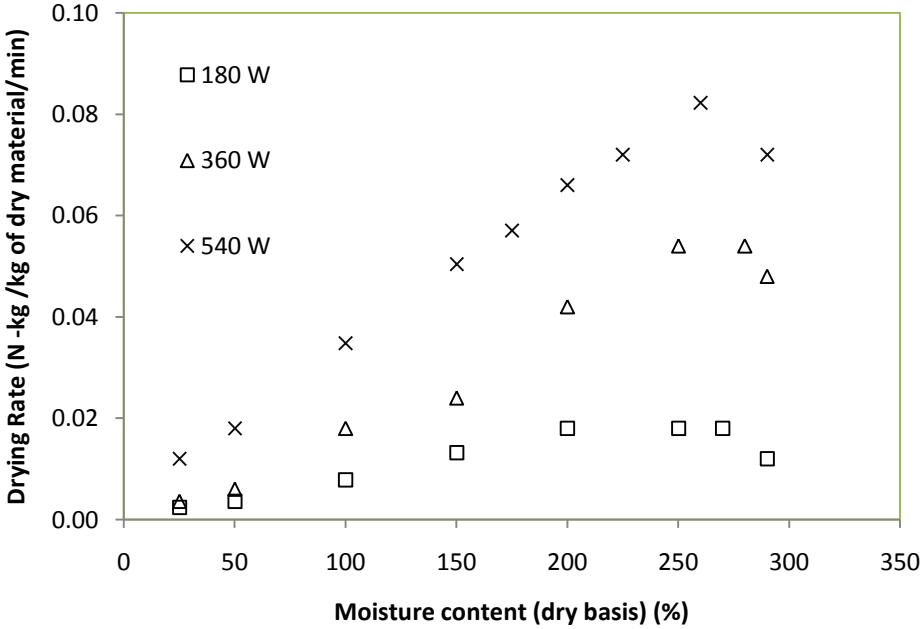


FIG 3: DRYING RATE AS A FUNCTION OF MOISTURE CONTENT FOR BLACK PEPPER DRYING AT THREE MICROWAVE POWER LEVELS

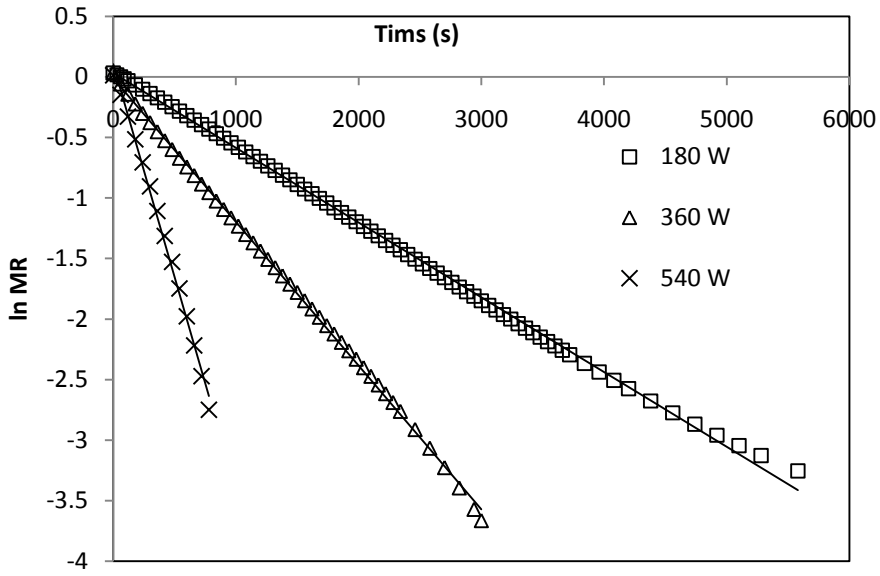


FIG 4: LN (MR) VALUES AS A FUNCTION TIME FOR THREE DIFFERENT MICROWAVE POWER LEVELS FOR BLACK PEPPER DRYING IN A MICROWAVE OVEN

TABLE 3
TIME REQUIRED FOR DRYING FROM 75 % – 11% MC WET BASIS, DRYING RATE CONSTANTS AND EFFECTIVE DIFFUSIVITY VALUES FOR BP DRYING IN A MICROWAVE OVEN

Power Level (W)	m/P (g/W)	Time required for drying (mins)	Drying rate constant, k, (Midilli *et. al* model) (min^{-1})	Effective Diffusivity (m^2/s)
180	0.0272	88	0.0312	2.43418E-10
360	0.0130	43	0.0955	4.86835E-10
540	0.0106	14	0.1603	1.41994E-09

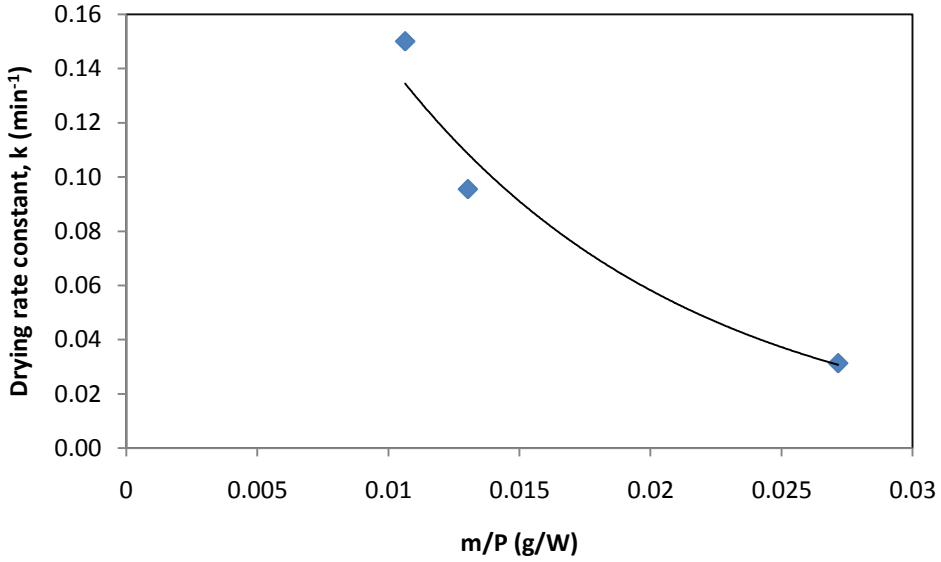


FIG 5: DRYING RATE CONSTANT, K, PREDICTED BY MIDILLI ET AL MODEL AS A FUNCTION OF THE RATIO OF WEIGHT OF BLACK PEPPER TO MICROWAVE POWER

V. CONCLUSION

Microwave drying kinetics of black pepper was studied and the experimental data was fitted to seven thin layer drying models. The Midilli *et. al.*, Weibull and Kaleta *et. al.* models showed statistically better fit compared to other models. Similarities of the model parameters were observed and the significance of the parameters was analyzed. The drying rate constants obtained by Midilli *et. al.*model were 0.03, 0.09 and 0.16 min^{-1} for power levels of 180, 360 and 540 W respectively and the other models also showed values in the similar range. The Effective moisture diffusivity increased with the microwave power level and values were in the range $2.43x\ 10^{-10}$- $1.42\ x\ 10^{-9}$ m^2/s. The drying rate constants and the effective diffusivity values were in the same order of magnitude as the values obtained by other workers for similar products. The Activation energy of black pepper calculated based on the Arrhenius equation is 86.7 W/g. Drying of BP under low microwave power level is recommended to preserve volatile matter in the product. The time required to dry peppercorns from 75% MC to 11% MC wet basis under 180 W power level is 88 minutes compared to several days under sun drying.

ACKNOWLEDGEMENTS

Authors wish to thank University of Moratuwa, Sri Lanka, for providing funds for the project.

REFERENCES

[1] NEDSPICE, Pepper Crop Report, Alfons van Gulick ESA Conference, Bordeaux, France,1st June 2017.

[2] National Priorities in Post harvest & Value Addition Research in Agriculture, 2011-2015National Committee on Postharvest Technology and Value Addition (NCPHT & VA),Sri Lanka Council for Agricultural Research Policy (SLCARP), Sri Lanka,(2011).

[3] H.Kucuk, A.Midilli, A. Kilic and I.Dincer, Review Article: A Review on Thin-Layer Drying-Curve Equations, , Drying Technology, 32, (2014), 757–773.

[4] O.A.Aregbesola, B.S.Ogunsina, A.E.Sofolahan and N.N. Chime, Mathematical modeling of thin layer drying characteristics of dika (Irvingiagabonensis) nuts and kernels, Nigerian food journal, 33 (2015) 83-89.

[5] A. Kaleta, K.Górnicki, R.Winiczenko and A. Chojnacka, Evaluation of drying models of apple (var. Ligol) dried in a fluidized bed dryer, Energy Conversion and Management, 67 (2013) 179–185.

[6] R. Sivakuar, R. Saravanan, A. ElayaPerumal and S. Iniyan, Fluidized bed drying of some agroproducts-A review, Renewable and sustainable Energy Reviews, 61, 2016 , 280-301.

[7] K.J. Chua and S.K. Chou, Low-cost drying methods for developing countries, Trends in Food Science and Technology, 14 (12), (2003) 519-528.

[8] D.I.Onwude, N. Hasim,R B Janius, N.M. Nawi and K Abdan, Modeling the Thin-layer Drying of Fruits and Vegetables:A Review, Comprehensive Reviews in Food Science and Food Safety, 15, 2016.

[9] M. Hemis, Y. Gariépy, R. Choudhary and V. Raghavan,New coupling model of microwave assisted hot-air drying of a capillaryporous agricultural product: Application on soybeans and canola seeds, Applied Thermal Engineering 114 (2017) 931–937.

[10] A. Balbay,Y.Kaya, O.Sahin, Drying of black cumin (Nigella sativa) in a microwave assisted drying systemand modeling using extreme learning machine, , Energy, 44 (2012) 352-357.

[11] A.C.da Silva, H. J. Sarturi, E. L. Dall'Oglio, M. A.Soares, P. T. de Sousa, L. G. de Vasconcelos and C. A.Kuhnen, Microwave drying and disinfestation of Brazil nut seeds, , Food Control 70 (2016) 119-129.

[12] A.S., Kassem, A.Z. Shokr, A.R. El-Mahdy, A.M.Aboukarima and E.Y.Hamed, Comparison of drying characteristics of Thompson seedless grapes using combined microwave oven and hot air drying, J.of the Saudi society of Agricultural Sciences, 10, (2001) 33-40.

[13] A. Magda, A.E.Mageed , A. F. Mansour , K, F. E.Massry , M. M. Ramadan and M. S.Shaheen, The Effect of Microwaves on Essential oils of White and Black Pepper (Piper nigrum L.) and their Antioxidant Activities, Journal of Essential Oil Bearing Plants, 14 (2), (2011) 214-223.

[14] C.M.I. Joy, G.P.Pittappilli and K.P. Jose, Drying of Black Pepper *(Piper nigrum*L.) Using Solar Tunnel Dryer, PertanikaJ. Trap. Agric. Sci. 25(1),(2002) 39 – 45.

[15] G.K. Jayatunga and B.M.W. P.K. Amarasinghe, Drying Kinetics of Black Pepper Dried in A Spouted Bed Dryer with or without Draft Tubes, International Journal of Manufacturing and Industrial Engineering (IJMIE), Vol. 1(2) June (2014), 6-10.

[16] S. Chacko, A. Jayalekshmy, M.Gopalakrishnan and C.S. Narayanan, Roasting studies on Black Pepper (Piper nigrum L.), Flavour and Fragrance journal, 11, (1996)305-310.

[17] I.Doymaz, Evaluation of some thin-layer drying models of persimmon slices (Diospyros kaki L.), Energy Conversion and Management, 56 (2012) 199–205.

[18] S. K. Pandey, S. Diwan and R Soni, Review of mathematical modeling of thin layer drying process, , online 2394-0697, 2 (11), 2015.

[19] Arun S.Mujumdar and S. Devahastin, Fundamental principles of Drying, Exergex, Brossard,Canada, (2000).

[20] H. Darvishia, A. R. Asl, A.Asghari, M.Azadbakht, ,G. Najafi and J.Khodaei,Study of the drying kinetics of pepper, Journal of the Saudi Society of Agricultural Sciences,13 (2014) 130–138.

[21] D. P. Rosa, D.Cantú-Lozano, G. Luna-Solano,T. C.Polachini and J.T.elis-Romero Mathematical modeling of orange seed drying kinetics,Ciênc. Agrotec.,Lavras, 39 (3), (2015) 291-300.

What do we know about adaptation to climate change in Africa? A review of grey literature

Oladayo Nathaniel Awojobi

Department of Social Security, Bonn-Rhein-Sieg University of Applied Sciences, Sankt Augustin, Germany

Abstract—

Background: *Africa is one of the regions that the impacts of climate change will be felt so much due to poor adaptive capacity and the reliance on agricultural production for human sustainability. While climate change is real in Africa, the continent has been building resilience through adaptation strategies.*

Objective: *To understand what adaptation strategies African countries have developed in response to and in anticipation of climate change.*

Methods: *The study reviews documents, reports and projects in the form of grey literature and content analysis was used to analyse these documents manually.*

Results: *Climate change has mostly affected the agricultural sector thereby reducing agricultural production. This has led to the introduction and implantation of adaptation policies and strategies on the African continent. However, there are barriers militating against adaptation measures.*

Conclusion: *In building resilience, some adaptation policies and strategies have been initiated mostly at the local levels with the financial support from donor agencies.*

Keywords: Adaptation, Africa, Climate change, Grey literature.

I. INTRODUCTION

When it comes to climate change, no one is immune to its impact, according to the Fuji Presidency of the Parties of the Conference (COP23) of the United Nations Framework Convention on Climate Change (UNFCCC). However, the degree of impacts varies across regions. Africa has been considered to be among the most vulnerable to the impacts of climate change (Association of German Development NGOs, 2009; UNDP, 2010; UNISDR, 2011; Ziervogel, Cartwright, et al., 2008). This was also noted in the Bali Action Plan acknowledged in Bali 2007, which acted as a major basis for the United Nations (UN) climate change negotiations at the Copenhagen climate summit (Association of German Development NGOs, 2009).

Drought, increasing temperatures, flooding and irregular rainfalls are some of the climate events that are common in Africa (see Awojobi & Tetteh, 2017). It is argued that these climate events have been responsible for forced migration, displacement and conflicts among Africans (Besada & Sewankambo, 2009).

Climate change is an element increasingly subverting development efforts (Association of German Development NGOs, 2009). Without adaptation, particularly in the agricultural sector, climate change will exacerbate the existing high level of poverty in the continent. This is because the agricultural sector is one of the main sources of employment generation.

Knowing fully well that Africa is considered as the most vulnerable to climate change. The continent since 2007, has gained know-how in conceptualizing, planning, and starting to execute and support adaptation activities (Niang et al., 2014). However, across Africa, most of the adaptation to climate change and variability is reactive in reaction to short-term motivations, is happening separately at the individual or household level, and lacks government support and policies (Berrang-Ford et al., 2011; Vermuelen et al., 2008; Ziervogel et al., 2008). As the climate change sphere unfolds(Ford et al., 2011). There is the need for new literature approaches if we are to establish and describe "what we know, don't know, and need to know" (Hulme, 2009; Hulme et al., 2010) as cited in (Ford et al., 2011).

In this paper, I develop a grey literature review methodology to understand what adaptation strategies African countries have developed in response to and in anticipation of climate change.

The content of the paper is structured as follows:

- Section 2 explains the methodology employs to understand African adaptation strategies. The paper relies solely on grey literature for the analysis.
- Section 3 describes the findings from the analysis of the grey literature that was used for data gathering.
- Section 4 draws on the conclusion and it uses the solutions to climate change adaptation in Africa from the grey literature as recommendations for the paper.

II. MATERIALS AND METHODS

2.1 Grey literature

Grey literature is "material that is not formally published by commercial publishers or peer-reviewed journals and is produced by institutions, academics, organizations and government agencies" (Degenhardt et al., 2016, p. 5). It comprises reports, conference proceedings, fact sheets and other documents from organizations, government agencies and institutions (Calabria et al., 2008). This paper decided to deviate from the normal peer-reviewed journals for secondary data collection and focuses on grey literature. The reason behind this is that climate change adaptation in Africa is mostly undertaken by the government and local and international organizations. Most of these organizations have the reports, policy and other documents published on their websites.

2.2 Searching for grey literature

The growth of the internet has made grey literature quickly accessible electronically in PDF format (Calabria et al., 2008). An electronic search was conducted on Google search engine with the topic "climate change adaptation in Africa pdf." The outcomes of the search produced a lot of literature both in peer-reviewed and grey literature. The grey literature was sorted out from the peer-reviewed articles. Some of the grey literature include reports, documents, studies, briefing note and projects.

Content analysis was used to analyse the data manually. Content analysis is a method ofanalysing documents and it "allows the researcher to test theoretical issues to enhance understanding of data" (Elo & Kyngäs, 2008, p. 108). Why content analysis has been criticized by some scholars, it is considered to bevery flexible in research design (Harwood & Garry, 2003).

2.3 Key findings

This section of the paper presents the findings from the grey literature that met the criteria of the study.

2.3.1 Climate change impacts in Africa

Without the impacts of climate change or future impacts, there will be no need for adaptation. The grey literature reviewed in this paper presents some of the climate change impacts in Africa. For instance, in South Africa, a study by the University of Pretoria found a positive correlation between higher temperatures and the reduction of dry land stable production that affected small-scale farmers (Ziervogelet al., 2008). In the southern African sub-region, El Niño associated with droughts that happened between 1965 and 1997led to the reduction in agricultural production (Chishakwe et al., 2012). Similarly, the United Nations International Strategy for Disaster Reduction reports that the famines that occurred in some parts of Africa in the late 1980s account for the greater part of the distress in relation to the number of casualties (Ethiopia – 300,000; Sudan – 150,000; Mozambique – 100,000; Somalia – 600) (UNISDR, 2011). While there is still continuing debate on the correlation between climate change and conflicts, the Association of German Development NGOs assert that the impacts of climate change pose a greater risk of conflict in various regions of Africa (Association of German Development NGOs, 2009).

2.3.2 Planning adaptation

Climate change will affect Africa now and in the future, according to environmental experts (see Association of German Development NGOs, 2009; UNISDR, 2011; Ziervogel, Taylor, et al., 2008). This has given the political leadership of the continent to take appropriate measures against the impacts of climate change. For example, Kenya has integrated climate change strategies in its national planning documents (FAO, 2014). In Senegal, a National Emergency Fund has been created for disaster risk reduction (UNISDR, 2011). Similarly, in Uganda, budgetary allocations are made yearly to the Department of Disaster Management based on its work plan for disaster risk reduction (UNISDR, 2011). Another model of such is in

place in Mozambique, where 53.9% of resources are committed to the ministry in charge of disaster management to fight disasters (UNISDR, 2011). These plans are to support climate change adaptation (UNISDR, 2011).

2.3.3 Practical adaptation responses

Some African countries have already taken practical steps in building climate resilience through adaptation measures. Most of these projects are supported by international development agencies who finance and designed these adaptation strategies. **Table 1** below lists some of these projects.

TABLE 1
ADAPTATION STRATEGIES IN SOME AFRICAN COUNTRIES

Adaptation Project	Country	Function
InforClim[1]	Senegal	To address gaps between adaptation practices of local government development plans
SADC Water Sector Climate Change Adaptation Strategy[2]	Southern African Development Community (SADC)	Aims at improving climate resilience in the region through integrated and adapted water resources management
FAO-sida intervention[3]	Ethiopia Kenya Tanzania	Adapting to climate change through land and water management in Easter Africa
Kyoto world water project[4]	Mauritania	To assist resolve water scarcity and sanitation problems

Sources: (Akoh et al., 2011[1]; Department of Environmental Affairs, n.d.[2]; FAO, 2014[3]; UNDP, 2010[4]).

2.3.4 Adaptation finance

Climate change adaptation is conveyed via a developing system of funds that composed of the Least Developed Countries Fund (LDCF), the Special Climate Change Fund (SCCF) and the Adaptation Fund (AF) (Chishakwe et al., 2012). Most of these funds are small in nature based on the voluntary pledges and donations from donors with the exclusion of the Adaptation Fund (Chishakwe et al., 2012). In Africa, various funds have been used to finance adaptation against climate change. The grey literature reviewed, listed some of the current climate funds significant in this paper. See **Table 2** below.

TABLE 2
ADAPTATION FINANCE AND PROJECTS IN AFRICA

Climate Project	Administered by	Total Pledged (US Dollar)
Mitigation Project in Kuyasa, South Africa[1]	Department of Environmental Affairs	US$ 3,76 million
Africa Biogas Partnership Programme[1]	Netherland	€30 million*
Water Security and Climate Resilient Development-Eritrea[1]	Adaptation Fund	US$ 6,5 million
Congo Forest Basin[2]	AfDB	US$165 million
Solar-powered water supply and irrigation system, Chanyauru, Tanzania[3]	UNDP	US$48,270 and US$4,541 (in two grants)
A community well and land reclamation, Hadiya village, Niger[3]	UNDP	US$18,509 and US$17,690 (in two grants)
Climate Change Adaptation in Africa[4]	AfDB	£24 million**

Sources: African Union, 2014[1]; Chishakwe et al., 2012[2]; UNDP, 2010[3]; Ziervogel, et al. 2008[4]
****The Project was financed in Euros***
***** The project was financed in British Pound Sterling***

2.3.5 What are the barriers to adaptation in Africa?

Africa recognizes the fact that adaptation is an overriding priority for the continent (African Union, 2014). While efforts have been concentrated in building resilience, there are barriers to adaptation in Africa. The grey literature in this paper illustrates some of these barriers. For instance, in Kenya, while the majority of the farmers are aware of climate change and its impacts,

they felt that their communities felt cut off from NGOs and government support about what adaptive strategies to embark on (Chishakwe et al., 2012). In the African agricultural sector, firstly, climate change data are not accessible at the spatial resolution needed by farmers and as such farmers battle to harmonize their observations of the weather with climate projections and lose confidence in the projections (Ziervogel et al., 2008). Secondly, the timeframes over which climate date are announced is not of much importance to farmers. While it is expected that policymakers should consider the implications of a 2050 projection, African farmers base their judgement on more immediate issues (Ziervogelet al., 2008). Finally, only a few African scientists have the requisite training and experience to interpret and apply climate change data in the agricultural context (Ziervogel et al., 2008).

III. CONCLUSIONS AND RECOMMENDATIONS

This grey literature review seeks to contribute to a better understanding of adaptation in Africa. While it is clear and certain that Africa has been considered as vulnerable to climate change from the reviewed literature, studies have shown some practical climate change impacts have affected mostly the agricultural sector. In building resilience, some adaptation policies and strategies have been initiated mostly at the local levels with the financial support from donor agencies. Despite African countries' attempts in building climate resilience through adaptation, there are still many barriers along the way. To address these situations, some recommendations have been suggested by the reviewed grey literature.

- The partnership between disaster risk reduction and climate change adaptation communities should be improved and institutionalized in order for African governments and donors to incorporate both disaster risk reduction and climate change adaptation concern into important public, private and household investment decision, based on the principles of cost-effectiveness and agreeable levels of risk to human life (UNISDR, 2011).
- Mainstreaming adaptation into national and regional economic and social development strategies, frameworks, and priorities will be important in reducing the impacts of climate change on sustainable development (USAID, 2012).
- Many African farmers know that climate change is real, however, many still see its impacts in the light of normal seasonal climate variability. A concerted effort is needed toraise enlightenment of climate change among farmers with the intensity of its implications for the choice of the farming methods, timing, and crop seed varieties (Chagutah, 2010).
- Capacity building and education have been used for agricultural innovation as ways of adapting to climate change impacts, laws and regulations should be drafted and enforced for sustainable use of land and water in communities (FAO, 2014).

REFERENCES

[1]. African Union. (2014). African strategy on climate change. Retrieved January 11, 2018, from http://cap.africa-platform.org/sites/default/files/resources/AMCEN-15-REF-11-%20Draft%20African%20Union%20strategy%20on%20climate%20change%20-%20English%20%281%29.pdf

[2]. Association of German Development NGOs. (2009). Adaption to Climate Change in the Joint Africa-EU Strategy and the Copenhagen Climate Summit. Germanwatch. Retrieved from https://germanwatch.org/klima/adafeucop.pdf

[3]. Awojobi, O. N., & Tetteh, J. (2017). THE IMPACTS OF CLIMATE CHANGE IN AFRICA: A REVIEW OF THE SCIENTIFIC LITERATURE. *Journal of International Academic for Multidisciplinary*, *5*(11), 39–52.

[4]. Berrang-Ford, L., Ford, J. D., & Paterson, J. (2011). Are we adapting to climate change? *Global Environmental Change*, *21*(1), 25–33. https://doi.org/10.1016/j.gloenvcha.2010.09.012

[5]. Besada, H., & Sewankambo, N. (2009). CIGI Special Report: Climate Change in Africa: Adaptation, Mitigation and Governance Challenges. The Centre for International Governance Innovation (CIGI). Retrieved from https://www.cigionline.org/sites/default/files/climate_change_in_africa_3.pdf

[6]. Calabria, B., Phillips, B., Singleton, J., Mathers, B., Congreve, E., Degenhardt, L., & McLaren, J. (2008). *Searching the grey literature to access information on drug and alcohol research: a resource to identify drug related databases and web sites*. Sydney, NSW, Australia: National Drug and Alcohol Research Centre, University of New South Wales.

[7]. Chagutah, T. (2010). Climate Change Vulnerability and Adaptation Preparedness in Southern Africa: Zimbabwe Country Report. Heinrich Böll Stiftung.

[8]. Chishakwe, N., Chambwera, M., & Murray, L. (2012). *Building climate change adaptation on community experiences lessons from community-based natural resource management in southern Africa*. London: International Inst. for Environment and Development.

[9]. Degenhardt, L., Gibson, G., Leung, J., Kumvaj, M., & Larney, S. (2016). Searching the grey literature to access research on illicit drug use, HIV and viral hepatitis. *NATIONAL DRUG AND ALCOHOL RESEARCH CENTRE, UNIVERSITY OF NEW SOUTH WALES, SYDNEY*.

[10]. Elo, S., & Kyngäs, H. (2008). The qualitative content analysis process. *Journal of Advanced Nursing*, *62*(1), 107–115. https://doi.org/10.1111/j.1365-2648.2007.04569.x

[11]. FAO (Ed.). (2014). *Adapting to climate change through land and water management in Eastern Africa: results of pilot projects in Ethiopia, Kenya and Tanzania*. Rome: Food and Agriculture Organization of the United Nations.

[12]. Ford, J. D., Berrang-Ford, L., & Paterson, J. (2011). A systematic review of observed climate change adaptation in developed nations: A letter. *Climatic Change*, *106*(2), 327–336. https://doi.org/10.1007/s10584-011-0045-5

[13]. Harwood, T. G., & Garry, T. (2003). An Overview of Content Analysis. *The Marketing Review*, *3*(4), 479–498. https://doi.org/10.1362/146934703771910080

[14]. Hulme, M. (2009). Mapping climate change knowledge: An editorial essay. *Wiley Interdisciplinary Reviews: Climate Change*, *1*(1), 1–8. https://doi.org/10.1002/wcc.3

[15]. Hulme, M., Zorita, E., F. Stocker, T., Price, J., & R. Christy, J. (2010). *IPCC: cherish it, tweak it or scrap it?* (Vol. 463). https://doi.org/10.1038/463730a

[16]. Niang, I., Ruppel, O., Abdrabo, M., Essel, A., Lennard, C., Padgham, J., & Urquhart, P. (2014). Africa. In: Climate change 2014: impacts, adaptation and vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge. Retrieved from https://www.ipcc.ch/pdf/assessment-report/ar5/wg2/WGIIAR5-Chap22_FINAL.pdf

[17]. UNDP. (2010). UNDP Community Water Initiative: Fostering Water Security and Climate Change Adaptation and Mitigation. United Nations Development Programme (UNDP).

[18]. UNISDR. (2011). Effective measures to build resilience in Africa to adapt to climate change. United Nations International Strategy for Disaster Reduction. Retrieved from http://www.unisdr.org/files/24012_briefingnote04africa.pdf

[19]. USAID. (2012). Climate Change Adaptation in Western Africa. USAID. Retrieved from https://www.climatelinks.org/sites/default/files/asset/document/west_africa_adaptation_fact_sheet_jan2012%20%281%29.pdf

[20]. Vermuelen, S., Dossou, K., Macqueen, D., Walubengo, D., & Nangoma, E. (2008). Spring back: climate resilience at Africa's grassroots. *IIED Report*.

[21]. Ziervogel, G., Cartwright, A., Tas, A., Adejuwon, J., Zermoglio, F., Shale, M., & Smith, B. (2008). Climate change and adaptation in African agriculture. *Stockholm Environment Institute*.

[22]. Ziervogel, G., Taylor, A., Hachigonta, S., & Hoffmaister, J. (2008). Climate adaptation in southern Africa: Addressing the needs of vulnerable communities. *Environment Institute (SEI), Stockholm, Sweden*. Retrieved from https://www.sei-international.org/publications.

Effect of Nitrobenzene granules and Seaweed extracts on biochemical contents of Arachis hypogaea callus culture.

S.R. Sivakumar[1], A.Nagaraj[2]

[1]Assistant Professor, Department of Botany, Bharathidasan University, Trichy-24, Tamilnadu, India.
[2]Department of Biotechnology, Bharathidasan University, Trichy-24, Tamilnadu, India.

Abstract— *The present study is aimed to evaluate the effect of organic extracts (benzene, diethyl ether and water) of seaweeds (*Halimeda *gracilis, Ceramium rubrum and Cystophyllum muricatum) and nitrobenzene granuleson biochemical contents of Arachishypogea L. callusunder in vitro conditions. The callus of Arachishypogea L. was obtained from the leaf explants on MS medium containing 2, 4-D (1 mgL^{-1}) and BAP (0.5 mg L^{-1}). The mass multiplication of callus was achieved at 1mg L^{-1} of 2, 4-D and 0.5 mg L^{-1} of GA_3. The calli were then treated with different concentrations (0.5, 1.0 and 1.5 mg L^{-1}) of seaweed extracts and Nitrobenzene granules. Total carbohydrate, total protein and total chlorophyll contents were analyzed at 5, 10 and 15 days intervals. The total carbohydrate content was high (3.7mg/100mg) in callus treated with Benzene extract of Ceramium rubrumat 1.5 mg L^{-1} on 15^{th} day. The total protein content was increased (6.9mg/100 mg) in callus treated with Benzene extract of Cystophyllum muricatum at 0.5 mg L^{-1} on 5^{th} day and the total chlorophyll content was lower (0.36mg/100mg) in Nitrobenzene granules at 0.5mg L^{-1} in 5^{th} day when compare to control. The present study reveals the positive role of different extracts of seaweeds on increasing the biochemical contents of callus culture of A.hypogea. The extracts can be further evaluated for their role on enhanced regeneration of plants from callus culture.*

Keywords— Nitrobenzene, Seaweed extract, Arachis hypogaea, **Halimeda** ***gracilis, Ceramium rubrum and Cystophyllum muricatum.***

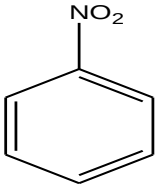
I. INTRODUCTION

Seaweed extracts are well-known biostimulants. The seaweed extract consist of trace elements and particularly plant growth regulators such as cytokinin, amino acids, antibiotics, and Vitamins. In modern trends the seaweeds were used along with the farm land as a soil conditioner in some European countries. There are different extraction methods can be used for seaweed extracts preparation i.e. water extraction under high pressure, alcohol extraction, alkaline extraction, microwave-assisted extraction (MAE) and supercritical CO_2 extraction. Cytokinins can be extracted using chilled 70% ethanol. Deuterium is used as co solvent in this process (Yokoya*et al.*,2010, Stirk*et al.,* 2009). Extraction in 85% methanol leads to obtainment of algae extract rich in gibberellins (Hytonen.*et al.,* 2009). Algal extracts improve plant resistance to frost and drought and increase crop yields. Plants sprayed with the use of seaweed extracts are also characterized by higher resistance to pests and pathogens and more efficient consumption of nutrients from soil (Matysiak K *et al*.,2010).

Groundnut (*Arachishypogaea* L.,) is one of the most important oil crops. The Groundnut provides major source of edible oil and vegetable protein. It contains 47-53% oil and 25-36% of protein. Groundnut is a self pollinated crop whereas flowers are produced above ground and after fertilization, pegs move towards the soil, and seed-contain pods are formed. The cultivated groundnut (*Arachishypogaea* L.,) originated in South America. China is the first country in cultivate groundnut and India is in second place. Peanut oil is often used in cooking, because it has a mild flavor and a relatively high smoke point. Due to its high monounsaturated content, it is considered healthier than saturated oils, and is resistant to rancidity. There are several types of peanut oil including: aromatic roasted peanut oil, refined peanut oil, extra virgin or cold pressed peanut oil and peanut extract. In the United States, refined peanut oil is exempt from allergen labeling laws. The top countries of peanut cultivars around the world in 2012.

Nitrobenzene is a greenish yellow crystal or yellow oily liquid with the odor of bitter. The nitrobenzene is soluble in water, acetone, benzene, diethyl ether, and ethanol. Nitrobenzene is applied with nitrogen for the enhancement of flowering and growth of agricultural crops. The nitrobenzene from seaweed extract has the capacity to increase flowering in plant (flower stimulant) and also prevent flower shedding.

1.1 Structure and Molecular formula:



$C_6H_5NO_2$

The science of tissue culture is historically linked to the discovery of the cell and subsequent propounding of the cell theory. Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. More than 234 years ago, Henri-Louis Duhamel du Monceau's(1756) pioneering experiments on wound healing in plants demonstrated spontaneous callus formation on the decorticated region of elm plants. The development of the multicellular or multiorganed body of a higher organism from a single-celled zygote supports the totipotent behaviour of a cell. . Haberlandt is regarded as the father of tissue culture. German botanist Gottlieb Haberlandt (1902) developed the concept of invitro cell culture. He was the first to culture isolated, fully differentiated cell in a nutrient medium containing glucose, peptone, and Knop's salt solution.

Callus, which shown stable characteristics under specific conditions after subculture through many successive passages, is a suitable material for cyto differentiation. The advantage of using such callus is that it is composed of fairly homogeneous mass cells and can be proliferated in large amounts under known culture conditions. Wetmore and Sorokin (1955) induced vascular strands in syringa callus derived from the cambial region of the stem or graft apices of shoots. Since then (1950) vascular or tracheary element differentiation has been induced in callus derived from tissues of many species. To Biostimulate the callus of *Arachishypogaea* L., using different seaweed extracts and it's biochemical assay. To collect seaweeds from Mandapam, Rameshwaram coastal Islands, Tamilnadu, India. To prepare seaweed extract by using three different organic solvents.To collect seed of *Arachis hypogaea* L. from Tamil Nadu Agriculture University Trichy,Tamilnadu,India.To prepare explants in half-strength MS Media.To induce callus in MS media with growth hormones.To Mass multiple the callus using growth hormones.To biostimulate callus by using crude seaweed extracts. To biostimulate callus by using nitrobenzene granules (Standard). To estimate the amount of total chlorophyll by Mackinney method. To estimate the amount of total carbohydrate by Anthrone method. To estimate the amount of total Protein by Lowery's method.

II. MATERIALS AND METHODS

2.1 Collection of Seaweeds:

The three different varieties of seaweeds such as *Halimeda gracilis*, *Ceramium rubrum* and *Cystophyllum muricatum*, were collected from Mandapam coastal Islands (Ramanathapuram District) Tamilnadu,India. Then the seaweeds were washed thoroughly with sea water to remove extraneous materials and brought to the laboratory in plastic bags containing water to prevent evaporation. Samples were then shade dried until constant weight obtained and ground in an electric mixer. The powder samples subsequently stored in refrigerator.

2.2 Preparation of seaweed extract:

The three seaweed were weighed 50 grams and extracted with 150 ml of diethyl ether, benzene and water each.

2.3 Collection of Seed:

The seed of *Arachis hypogaea* L., TMV-7 was collected from Tamilnadu Agriculture University Trichy, Tamilnadu,India.

2.4 Preparation of Explant:

The seed were surface sterilized by using 0.1% mercuric chloride and ethanol and it was inoculated in the half-strength MS medium. This was incubated in 12 hrs light and 12 hrs dark conditions at 25^0 C for 8 to 10 days.

2.5 Induction of Callus:

The explants were collected and that surface were damaged and inoculated in the MS medium and this was incubated in 12 hrs light and 12 hrs dark conditions at 25^0 C for 10 to 15 days.

2.6 Bio stimulation of Callus by using seaweed extract:

The callus was treated with three seaweed extracted with three different solvent in three different concentrations such as 0.5, 1.0 & 1.5 mg/l in three different incubation time such as (5, 10 & 15 days) and was incubated in 12 hrs light and 12 hrs dark conditions at 25°C under aseptic condition.

2.7 Biostimulation of Callus by using Nitrobenzene:

The callus was treated with nitrobenzene purchased from Greenland Bio-science Vadodara, Gujarat,India in three different concentration 0.5,1 & 1.5 mg/l.

2.8 Estimation of Chlorophyll by Mackinney 1941:

2.8.1 Procedure:

20 mg of each callus was taken and ground separately using 80% acetone (the volume make up to 2 ml).The extract was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and absorbance was read at 645nm and 663nm against the blank (80% acetone).

2.8.2 Calculation:

Use Arnon's equation (below) to convert absorbance measurements to mg Chl g-1 leaf tissue.

Chl a (mg g-1) = [(12.7 × A663) - (2.6 × A645)] × ml acetone / mg leaf tissue/1000.

Chl b (mg g-1) = [(22.9 × A645) - (4.68 × A663)] × ml acetone / mg leaf tissue/1000.

Total Chl = Chl a + Chl b.

2.9 Estimation of Carbohydrate by Anthrone Method (Hansen J, Møller IB, 1975) :

Dissolve 0.2g of anthrone in 5 ml of ethanol Add slowly 75%of sulphuric acid till the mark reaches 100 ml in standard measuring flask.

2.9.1 Procedure:

20 mg of callus wash taken and that was ground by using 1ml of distilled water this was took as test sample. Keep the test tube in an ice bath and slowly add 5 ml of the cold Anthrone reagent and mix properly. Close the test tubes with aluminium foil and place it in a boiling water bath for 10 min. Cool the tubes and measure OD at 620 nm. Blank should be prepared as per previous steps without adding test solution.

2.9.2 Observation:

Green color formation is noted.

2.10 Estimation of Protein Lowery's Method et al 1940:

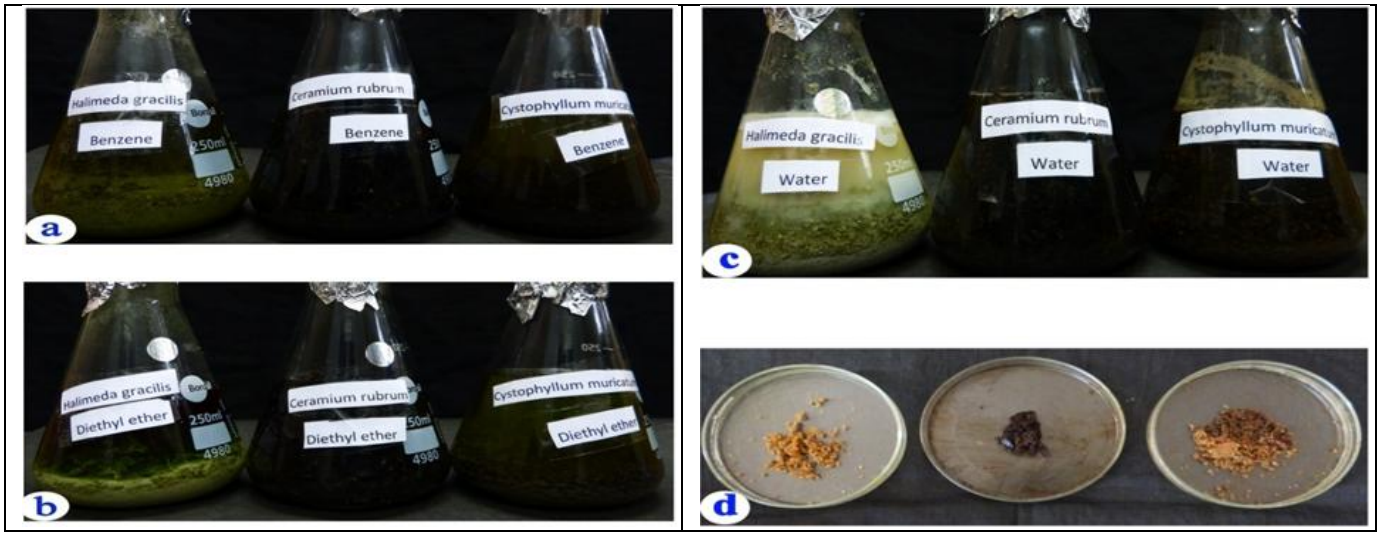
2.10.1 Procedure:

20 mg of callus wash taken and that was ground by using 1ml of distilled water from this 0.2ml was took as test sample. To each test tube add 1 mL of the mixed reagent and mix thoroughly and allow to stand at room temperature for 10 min or longer. Add 0.3 mL of diluted Folin-Ciocolteau reagent rapidly and mix properly. Incubate all tubes for 60 minutes. Measure OD of the standard and test solution at 660nm and plot the standard graph. Run the blank.

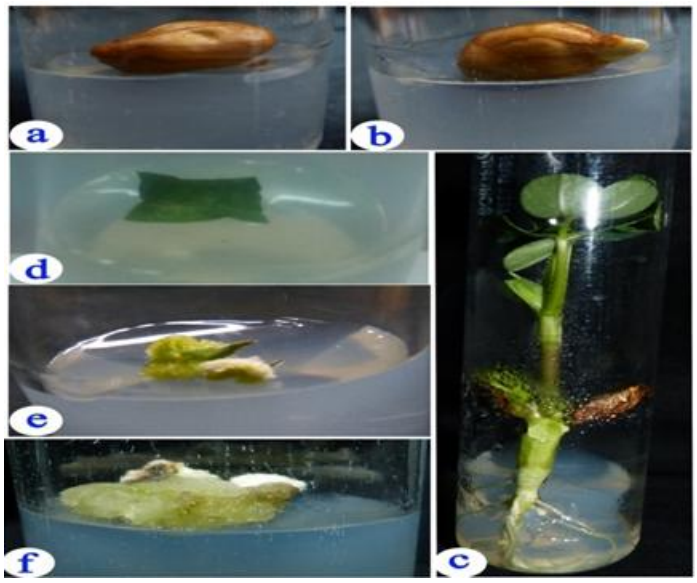
2.10.2 Observation:

Blue colour is noted and read using spectrophotometer.

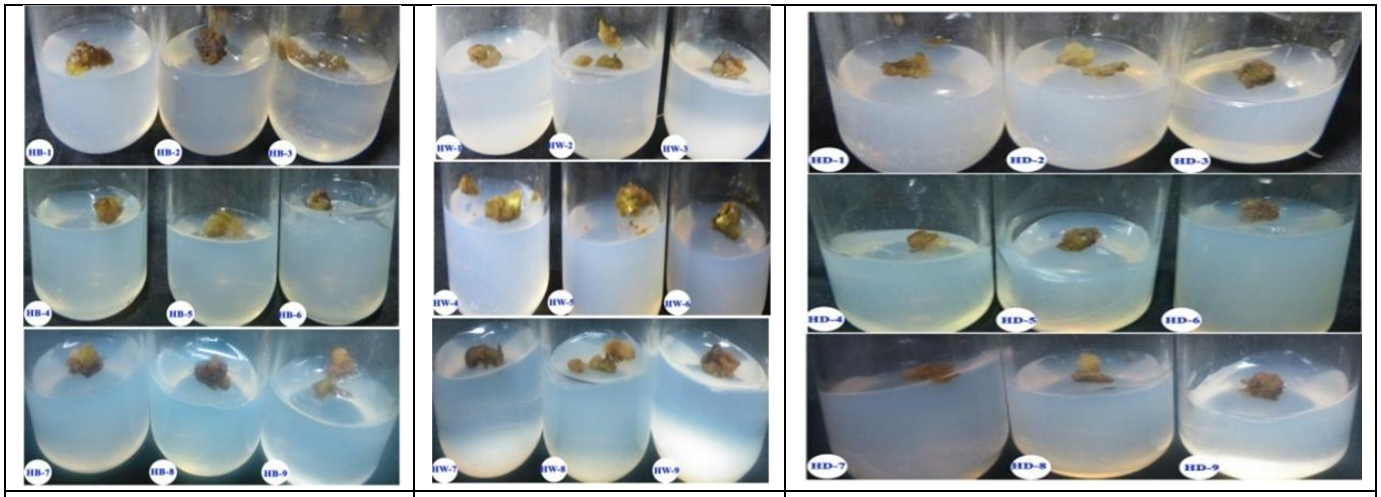
2.11 Extraction of Seaweed in Solvents



2.11.1 CALLUS INDUCTION OF *Arachis hypogiea L.*

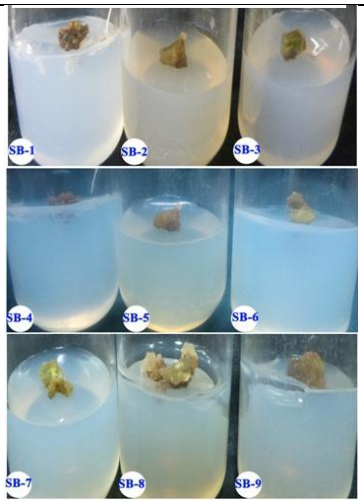
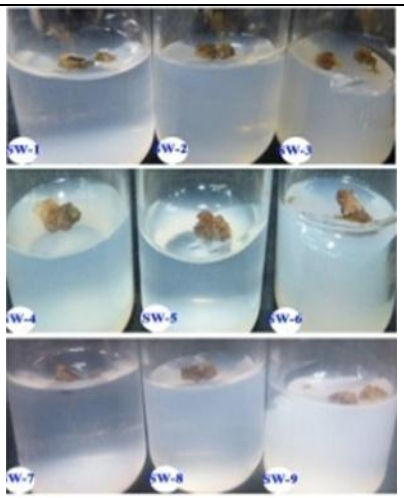
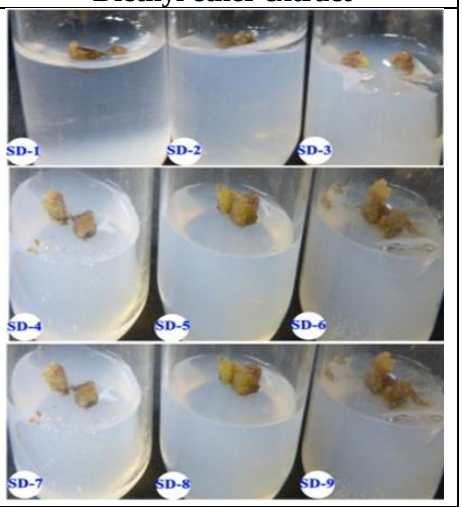


2.11.2 Callus treatments of Halimeda gracillis at different concentrations:

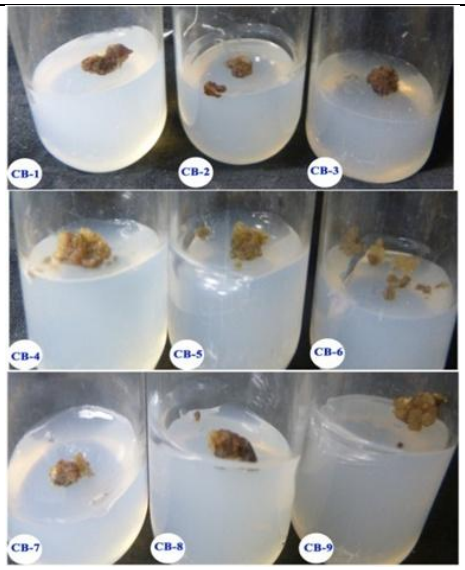
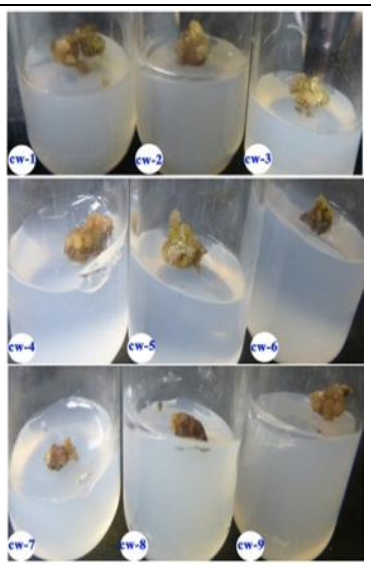
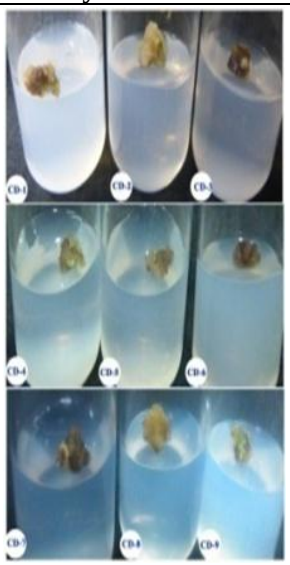


Benzene extract	Water extracxt	Diethyl ether extract

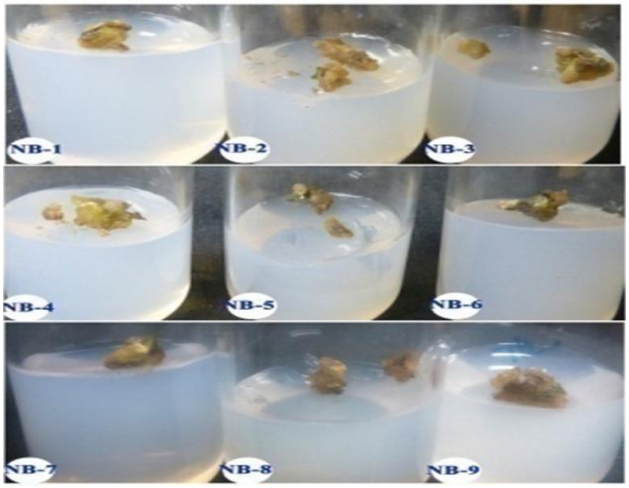
2.11.3 Callus treatments of *Halimeda gracillis* at different concentrations:

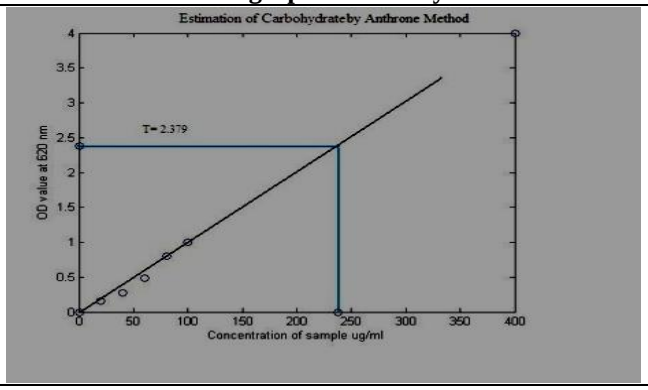
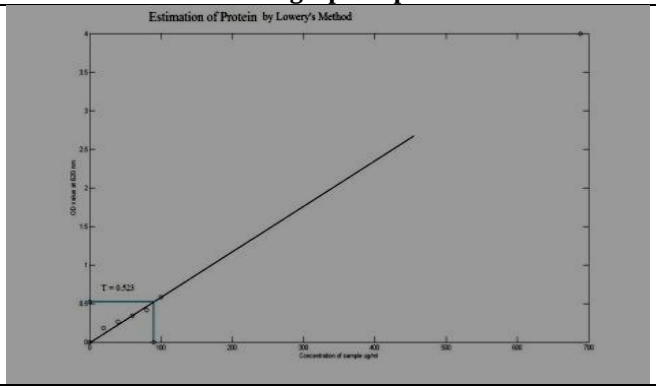
Benzene extract	Water extracxt	Diethyl ether extract
		

2.11.4 Callus treatments of *Cystopyllum muricatum* at different concentrations:

Benzene extract	Water extracxt	Diethyl ether extract
		

2.11.5 Callus treated with Nitrobenzene granules



Standard graph of carbohydrate	Standard graph of protein
	

III. RESULT AND DISCUSSION

3.1 Extraction of seaweeds in solvents:

A total of three seaweeds were extracted with three different organic solvents and incubated for 25 days at room temperature. The solvent extracts were air evaporated slowly and the aqueous extract was dried in hot air oven. After evaporation, the sample was weighed and used as standard stock. Extracts of three seaweeds with different organic solvents are shown in Fig; 5.1a-d. All the extracted samples were weighed after evaporation and their list are given in **Table 4**. There are lot of methods used for seaweed extraction *Saragassum wightii, Gelidella aerosa* and *Ulva lactuca* extracted with 5% acetone (Immanuel and Subramanian, 1999). *Saragassum plgiophyllum* was extracted by soaked and boiled (Anantharaj and venkatasalu, 2001 and Ashok et al., 2004). *Saragassum polycystum, Ulva lactuca* and *Tubinaria conoides* were extracted with 50% ethyl alcohol (Ramamoorthy and Sujatha 2007). In present study, seaweeds were extracted with 100% Benzene and Diethyl ether and Water. Solvents were selected on the basis of polarity. Liquid extract of *Saragassum wightii* increased height and number of branches in *Arachis hypogaea* under field condition when compared to chemical treatment (Sridhar and Rengasamy 2010). Seaweed extract not only increased the vegetative growth of the plant but it also triggers the early flowering and fruiting in crops. Another experiment conducted by Zodape et al., 2008 showed that treatment of seaweed extract increased shoot length (31.7%), diameter (18.2%) and yield (37.4%) of *Abelmoschus esculentus* than the control. There is no previous study on treatment of callus with seaweed extracts.

3.2 Callus induction in Arachis hypogaea L.

The surface sterilized peanut seeds were inoculated in half strength MSB_5 medium (**Fig: 4 a**). The seeds germinated after 3-4 days (**Fig: 4 b**), the seedling grown after 10-12 days (**Fig: 4 c**). From this the leaf was chosen as explants and inoculated in MSB_5 medium containing 2,4, D and BAP at various concentration was shown in **(Fig: 4 d).** The maximal level of callus was obtained on MSB_5 medium supplemented with 2,4, D(1 mg L^{-1}) and BAP(0.5 mg L^{-1}) at 10-12 days(**Fig 4 e**). The callus induced on MSB_5 media was green in colour. Mass multiplication of the induced callus was achieved on MSB_5 medium containing 2,4, D (1 mg L^{-1}) and GA_3 (0.5 mg L^{-1}) (**Fig: 4 f**). Callus of *A.hypogea* was induced by using different concentration of 2, 4, D. The 2 mg / L 2,4-D gives maximum level of callus (Alam and Khaleque 2010). The callus from hypocotyls explants were obtained in 0.5 mg/ L of 2, 4-D along with BAP (Muthusamy et al.,2007). Maximum level of callus from mature tissues was obtained from 3.0 mg /L of IAA and 1.0 mg /L of BAP (Plalanivel et al., 2000). In our studies the explants was treated with 2,4-D alone, BAP alone and combination of 2,4-D with BAP at various concentration the maximal level of callus was obtained in (1 mg L^{-1}) of 2,4-D and (0.5 mg L^{-1}) of BAP. The mass multiplication was achieved in (1 mg L^{-1}) of 2,4-D and (0.5 mg L^{-1}) of GA_3.

3.3 Treatment of callus with Benzene extract of *Halimeda gracillus* and its biochemical assay

MS medium supplemented with Benzene extract of *Halimeda gracillus* as bio stimulant in place of plant growth regulators at various concentrations (0.5, 1.0 &1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5,10 & 15 days) (**Fig : 5**). Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1,** total chlorophyll estimation values is given in **Table 2**, The bar diagram representation of total chlorophyll estimation is shown in (**Fig : 16 a**). The total carbohydrate and total protein values is in **Table 3 ,** The total carbohydrate bar diagram representation in (**Fig :17 a**) and the total protein bar diagram representation in (**Fig :18 a**). Among

them, HB-3 showed 0.33 mg/ 100 mg and the minimum level is HB-1 showed 0.07 mg/100 mg. This result indicates that total chlorophyll content is lowering treated callus tissues when compared to control HB-7 showed 3.8 mg/100 mg and the minimum level in HB-5 showed 2.2 mg/ 100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. HB-8 showed 3.7 mg/ mg and the minimum level is HB-6 showed 0.8 mg / 100 mg. This result indicates that the protein content is higher in treated callus tissues when compared to control.

3.4 Treatment of callus with aqueous extract of *Halimeda gracilus* and its bio chemical assay

MS medium supplemented with aqueous extract of *Halimeda gracilus* as bio stimulant in place of plant growth regulators at various concentrations (0.5, 1.0 & 1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5,10 &15 days) **(Fig:6).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig: 16 b)**. The total carbohydrate and total protein value is given in **Table 3**, the total carbohydrate bar diagram representation in **(Fig: 17 b)** and the total protein bar diagram representation in **(Fig: 18 b).** Among them, HW-3 showed 0.34 mg/100mg and the minimum level is HW-8 showed 0.29 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissues when compared to control. HW-7 showed 3.5 mg/100 mg and the minimum level is HW-6 showed 2.1 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. HW-7 showed 6.3 mg/100 mg and the minimum level is HW-5 showed 0.5 mg/ 100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.5 Treatment of callus with Diethyl ether extract of *Halimeda gracilus* and its biochemical assay

MS medium preparation with Diethyl ether extract of *Halimeda gracilus* as bio stimulant in place of plant growth regulators at various concentrations (0.5, 1.0 & 1.5) mg L^{-1} used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 7).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table: 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 17 f)** and the total protein bar diagram representation in **(Fig: 18 f)**. Among them, SD-7 showed 0.33mg/100 mg and the minimum level is SD-3 showed 0.020 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. SD-9 showed 3.4 mg/ 100 mg and the minimum level is SD-6 showed 2.2mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. SD-8 showed 3.8 mg/100 mg and the minimum level is SD-5 showed 0.5 mg/ 100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.6 Treatment of callus with benzene extract of *Ceramium rubrum* and its biochemical assay

MS medium supplemented with benzene extract of *Ceramium rubrum* as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 8).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 16 d).** The carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 d)** and the total protein bar diagram representation in **(Fig: 18 d)**. Among them, SB-3 showed 0.35mg/100 mg and the minimum level is SD-7 showed 0.30 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. SB-9 showed 3.7 mg/100 mg and the minimum level is SB-5 showed 2.1 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. SB-7 showed 4.2 mg/ 100 mg and the minimum level is SB-5 showed 0.6 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.7 Treatment of callus with aqueous extract of *Ceramium rubrum* and its biochemical assay

MS medium supplemented with aqueous extract of *Ceramium rubrum* as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 9).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of

total chlorophyll estimation is shown in **(Fig 16 e).** The total carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 e)** and the total protein bar diagram representation in **(Fig: 18 e)**. Among them, SW-6 showed 0.38mg/100 mg and the minimum level is SW-8 showed 0.27 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. SB-8 showed 3.1 mg/100 mg and the minimum level is SW-6 showed 2.3 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. SB-8 showed 6.8 mg/ 100 mg and the minimum level is SW-5 showed 0.6 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.8 Treatment of callus with diethyl ether extract of *Ceramium rubrum* and its biochemical assay

MS medium supplemented with diethyl ethern extract of *Ceramium rubrum* as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 10).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 16 f).** The total carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 f)** and the total protein bar diagram representation in **(Fig: 18 f)**. Among them, SD-7 showed 0.33mg/100 mg and the minimum level is SD-3 showed 0.20 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. SD-9 showed 3.4 mg/100 mg and the minimum level is SD-6 showed 2.2 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. SD-8 showed 3.8 mg/ 100 mg and the minimum level is SD-5 showed 0.5 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.9 Treatment of callus with benzene extract of *Cystophyllum muricatum* and its biochemical assay

MS medium supplemented with benzene extract of *Cystophyllum muricatum* as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 11).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 16 g).** The total carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 g)** and the total protein bar diagram representation in **(Fig: 18 g)**. Among them, CB-6 showed 0.35mg/100 mg and the minimum level is CB-3 showed 0.07 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. CB-7 showed 3.7mg/100 mg and the minimum level is CD-6 showed 2.6 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. CB-7 showed 6.9 mg/ 100 mg and the minimum level is CB-4 showed 1.4 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.10 Treatment of callus with aqueous extract of *Cystophyllum muricatum* and its biochemical assay

MS medium supplemented with aqueous extract of *Cystophyllum muricatum* as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 12).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 16 h).** The total carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 h)** and the total protein bar diagram representation in **(Fig: 18 h)**. Among them, CW-2 showed 0.35mg/100 mg and the minimum level is CB-7 showed 0.27 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. CW-9 showed 3.1mg/100 mg and the minimum level is CW-4 showed 2.2 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. CB-8 showed 4.2 mg/ 100 mg and the minimum level is CB-5 showed 0.4 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.11 Treatment of callus with diethyl ether extract of Cystophyllum muricatum and its biochemical assay

MS medium supplemented with diethyl ether extract of*Cystophyllum muricatum* as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 13).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 16 i).** The total carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 i)** and the total protein bar diagram representation in **(Fig: 18 i)**. Among them, CD-2 showed 0.34mg/100 mg and the minimum level is CD-8 showed 0.25 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. CD-7 showed 3.1mg/100 mg and the minimum level is CD-6 showed 2.1 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. CD-9 showed 4.2 mg/ 100 mg and the minimum level is CD-6 showed 1.0 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.12 Treatment of callus with Nitrobenzene granules and its biochemical assay

MS medium supplemented with Nitrobenzene granules as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 14).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 16 j).** The total carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 j)** and the total protein bar diagram representation in **(Fig: 18 j)**. Among them, NB-3 showed 0.29mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. NB-9 showed 3.6mg/100 mg and the minimum level is NB-4 showed 2.0 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. NB-8 showed 3.7 mg/ 100 mg and the minimum level is NB-4 showed 0.6 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

Seaweed machinery such as macro- and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid like growth substance have an effect on cellular metabolism in treated plants leading to enhanced growth and crop yield(Crouch and others 1992; Crouch and van Staden 1993; Reitz and Trumble 1996; Durand and others 2003; Stirk and others 2003; Ordog and others 2004). Seaweed and seaweed-derived products has commonly used as amendments in crop production systems due to the attendance of a quantity of plant growth-stimulating compound (Wajahatullah Khan *et al*., 2009). Biostimulants are defined as ‘‘materials, other than fertilizers, that promote plant growth when applied in small quantities’’ and are also referred to as ‘‘metabolic enhancers. (Zhang and Schmidt 1997) The biostimulant present in seaweed extract increase the vegetative growth (10%), the leaf chlorophyll content (11%), the stomata density (6.5%), photosynthetic rate and the fruit production (27%) of the plant (Spinelli *et al*., 2010). Seaweed extractsimplies the presence of more than one group of plant growth-promoting substances/hormones (Tay and others 1985; Crouch and van Staden 1993). Cytokinins have been detected in fresh seaweeds (Hussein and Boney 1969) and seaweed extracts (Brain and others 1973).Marine algae are also hypothetically rich in auxins and auxin-like compounds (Crouch and van Staden 1993). Chemical analysis of the aqueous extract of (*Padina pavonica*) showed the attendance of macronutrients such as nitrogen (N), phosphorus (P) and potassium (K) required for maturity and growth of plants (Asma Chbani 2013). A chain of poly pheonolic compounds, flavonoids, flavonol glycosides have been identified from methanol extract of red and brown algae (Santoso *et al*., 2002). The HPLC analysis of *Ulva lactuca.* L in acetone extract shown high amount of phenolic compound (Vanillin, p-coumaric acid are major compounds) (Hassan and Ghareib 2009). The cytokinine such as Trans-zeatin, dihydrozeatin and iso-pentenyladenosine has been identified from *Fucus serratus* L. by GC-MS analysis.(Stirk and Van Staden 1997). *Kappaphycus alvarezii*, seaweed has been reported to be various organic extracts has confirmed the presence of the plant growth regulators (PGRs) Indole 3-acetic acid, Gibberellin GA_3, Kinetin, and Zeatin (Kamalesh prasad *et al*., 2010). Recent research suggests that application of seaweed extract as seed treatment and/or foliar spray helps significant growth of plants. The extract contains micro-nutrients, auxins and cytokinins and other growth promoting substances (Spinelli *et al*., 2010).

TABLE 1
DETERMINATION OF CALLUS WEIGHT IN THREE SEAWEEDS AT THREE DIFFERENT SOLVENT EXTRACTIONS, IN THREE CONCENTRATIONS UPTO 15TH DAY.

	Control mg	5 days			10 days			15 days		
Halimedagracilis		0.5 mg-L	1 mg-L	1.5 mg-L	0.5 mg-L	1 mg-L	1.5 mg-L	0.5 mg-L	1 mg-L	1.5 mg-L
Benzene	20	HB-1 95	HB-2 92	HB-3 69	HB-4 61	HB-5 56	HB-6 65	HB-7 135	**HB-8 158**	HB-9 98
H_2O	20	HW-1 82	HW-2 92	HW-3 67	HW-4 61	HW-5 56	HW-6 62	HW-7 63	**HW-8 84**	HW-9 60
Di ethyl ether	20	HD-1 41	HD-2 63	HD-3 62	HD-4 56	HD-5 63	HD-6 63	HD-7 42	**HD-8 66**	HD-9 44
Ceramiumrubrum										
Benzene	20	SB-1 78	SB-2 78	SB-3 72	SB-4 52	SB-5 98	SB-6 102	SB-7 80	SB-8 63	**SB-9 86**
H_2O	20	SW-1 50	SW-2 47	SW-3 60	SW-4 75	SW-5 87	SW-6 59	**SW-7 95**	SW-8 85	SW-9 71
Di ethyl ether	20	SD-1 110	SD-2 92	SD-3 85	SD-4 120	SD-5 110	**SD-6 130**	SD-7 51	SD-8 88	SD-9 98
Cytophyllummuricatum										
Benzene	20	CB-1 55	CB-2 60	CB-3 77	**CB-4 97**	CB-5 48	CB-6 79	CB-7 83	CB-8 58	CB-9 48
H_2O	20	CW-1 102	CW-2 109	CW-3 113	CW-4 43	CW-5 77	**CW-6 120**	CW-7 57	CW-8 60	CW-9 106
Di ethyl ether	20	CD-1 92	**CD-2 98**	CD-3 84	CD-4 95	CD-5 77	CD-6 84	CD-7 92	CD-8 76	CD-9 92
Nitrobenzene	**20**	NB-1 73	NB-2 83	NB-3 75	NB-4 112	NB-5 62	NB-6 78	NB-7 96	**NB-8 113**	NB-9 108

TABLE 2
CHLOROPHYLL A AND B OD VALUES AND CONCENTRATION

Samples	OD at 663	OD at 645	Chl a ug/ ml	Chl b ug/ml	Total Chl mg/100mg
HB1	0.073	0.047	0.040245	0.036733	0.076978
HB2	0.266	0.223	0.13992	0.193091	0.333011
HB3	0.254	0.23	0.13139	0.203914	0.335304
HB4	0.221	0.206	0.113555	0.184156	0.297711
HB5	0.238	0.218	0.12279	0.193918	0.316708
HB6	0.206	0.196	0.10533	0.176216	0.281546
HB7	0.243	0.217	0.126095	0.191603	0.317698
HB8	0.204	0.201	0.10341	0.182409	0.285819
HB9	0.246	0.216	0.12813	0.189756	0.317886
HW1	0.251	0.227	0.129875	0.201181	0.331056
HW2	0.228	0.22	0.11618	0.198548	0.314728
HW3	0.285	0.224	0.151855	0.18979	0.341645
HW4	0.241	0.217	0.124825	0.192071	0.316896
HW5	0.226	0.207	0.1166	0.184131	0.300731
HW6	0.235	0.208	0.122185	0.18317	0.305355
HW7	0.228	0.21	0.11748	0.187098	0.304578
HW8	0.222	0.205	0.11432	0.182777	0.297097
HW9	0.264	0.223	0.13865	0.193559	0.332209
HD1	0.267	0.223	0.140555	0.192857	0.333412

HD2	0.232	0.209	0.12015	0.185017	0.305167
HD3	0.195	0.185	0.099775	0.166195	0.26597
HD4	0.255	0.225	0.132675	0.197955	0.33063
HD5	0.262	0.228	0.13673	0.199752	0.336482
HD6	0.24	0.217	0.12419	0.192305	0.316495
HD7	0.223	0.21	0.114305	0.188268	0.302573
HD8	0.219	0.202	0.112805	0.180044	0.292849
HD9	0.189	0.181	0.096485	0.163019	0.259504
SB1	0.236	0.211	0.12243	0.186371	0.308801
SB2	0.268	0.224	0.14106	0.193768	0.334828
SB3	0.288	0.24	0.15168	0.207408	0.359088
SB4	0.238	0.217	0.12292	0.192773	0.315693
SB5	0.275	0.232	0.144465	0.20129	0.345755
SB6	0.252	0.224	0.1309	0.197512	0.328412
SB7	0.245	0.217	0.127365	0.191135	0.3185
SB8	0.254	0.219	0.13282	0.191319	0.324139
SB9	0.263	0.213	0.139315	0.182343	0.321658
SW1	0.244	0.209	0.12777	0.182209	0.309979
SW2	0.223	0.203	0.115215	0.180253	0.295468
SW3	0.249	0.216	0.130035	0.189054	0.319089
SW4	0.228	0.211	0.11735	0.188243	0.305593
SW5	0.242	0.216	0.12559	0.190692	0.316282
SW6	0.329	0.253	0.176025	0.212699	0.388724
SW7	0.234	0.211	0.12116	0.186839	0.307999
SW8	0.197	0.194	0.099875	0.176032	0.275907
SW9	0.293	0.24	0.154855	0.206238	0.361093
SD1	0.226	0.211	0.11608	0.188711	0.304791
SD2	0.204	0.199	0.10367	0.180119	0.283789
SD3	0.161	0.14	0.084035	0.122626	0.206661
SD4	0.22	0.203	0.11331	0.180955	0.294265
SD5	0.227	0.212	0.116585	0.189622	0.306207
SD6	0.242	0.224	0.12455	0.199852	0.324402
SD7	0.254	0.231	0.13126	0.205059	0.336319
SD8	0.192	0.179	0.09865	0.160027	0.258677
SD9	0.239	0.215	0.123815	0.190249	0.314064
CB1	0.221	0.205	0.113685	0.183011	0.296696
CB2	0.25	0.218	0.13041	0.19111	0.32152
CB3	0.03	0.066	0.01047	0.06855	0.07902
CB4	0.24	0.217	0.12419	0.192305	0.316495
CB5	0.203	0.222	0.100045	0.206688	0.306733
CB6	0.286	0.238	0.15067	0.205586	0.356256
CB7	0.23	0.211	0.11862	0.187775	0.306395
CB8	0.255	0.221	0.133195	0.193375	0.32657
CB9	0.199	0.217	0.098155	0.201899	0.300054
CW1	0.261	0.226	0.136355	0.197696	0.334051
CW2	0.262	0.251	0.13374	0.226087	0.359827
CW3	0.255	0.222	0.133065	0.19452	0.327585
CW4	0.214	0.203	0.1095	0.182359	0.291859
CW5	0.204	0.197	0.10393	0.177829	0.281759
CW6	0.233	0.213	0.120265	0.189363	0.309628
CW7	0.211	0.192	0.109025	0.170466	0.279491
CW8	0.216	0.191	0.11233	0.168151	0.280481
CW9	0.203	0.198	0.103165	0.179208	0.282373
CD1	0.216	0.2	0.11116	0.178456	0.289616
CD2	0.259	0.239	0.133395	0.213049	0.346444
CD3	0.244	0.209	0.12777	0.182209	0.309979
CD4	0.226	0.2	0.11751	0.176116	0.293626
CD5	0.26	0.226	0.13572	0.19793	0.33365

CD6	0.271	0.228	0.142445	0.197646	0.340091
CD7	0.22	0.208	0.11266	0.18668	0.29934
CD8	0.195	0.179	0.100555	0.159325	0.25988
CD9	0.199	0.206	0.099585	0.189304	0.288889
NB1	0.316	0.234	0.17024	0.193986	0.364226
NB2	0.276	0.235	0.14471	0.204491	0.349201
NB3	0.231	0.202	0.120425	0.177236	0.297661
NB4	0.241	0.221	0.124305	0.196651	0.320956
NB5	0.249	0.227	0.128605	0.201649	0.330254
NB6	0.243	0.228	0.124665	0.204198	0.328863
NB7	0.244	0.219	0.12647	0.193659	0.320129
NB8	0.24	0.222	0.12354	0.19803	0.32157
NB9	0.228	0.208	0.11774	0.184808	0.302548

TABLE 3
CARBOHYDRATE AND PROTEIN OD VALUE AND CONCENTRATION

	CARBOHYDRATE		PROTIEN	
	OD at 620nm	100mg/mG	OD at 660nm	100mg/g
HB-1	2.635	2.635	0.371	3.2
HB-2	2.352	2.352	0.429	3.7
HB-3	2.469	2.469	0.184	1.6
HB-4	2.529	2.529	0.084	0.7
HB-5	2.271	2.271	0.099	0.8
HB-6	2.215	2.215	0.077	0.6
HB-7	3.496	3.496	0.214	1.8
HB-8	3.082	3.082	0.170	1.4
HB-9	2.492	2.492	0.085	0.7
HW-1	2.811	2.811	0.729	6.3
HW-2	2.670	2.670	0.315	2.7
HW-3	2.809	2.809	0.330	2.8
HW-4	2.135	2.135	0.152	1.3
HW-5	2.800	2.800	0.066	0.5
HW-6	2.158	2.158	0.163	1.4
HW-7	3.557	3.557	0.347	3.0
HW-8	3.533	3.533	0.326	2.8
HW-9	2.538	2.538	0.230	2.0
HD-1	2.717	2.717	0.319	2.7
HD-2	2.352	2.352	0.557	4.8
HD-3	2.469	2.469	0.357	3.1
HD-4	2.529	2.529	0.064	0.5
HD-5	2.271	2.271	0.135	1.1
HD-6	2.215	2.215	0.105	0.9
HD-7	3.496	3.496	0.194	1.7
HD-8	3.082	3.082	0.421	3.6
HD-9	2.492	2.492	0.264	2.3
SB-1	2.773	2.773	0.485	4.2
SB-2	2.728	2.728	0.292	2.5
SB-3	2.785	2.785	0.342	2.9
SB-4	2.516	2.516	0.109	0.9
SB-5	2.119	2.119	0.070	0.6
SB-6	2.270	2.270	0.135	1.1
SB-7	3.617	3.617	0.306	2.6
SB-8	3.134	3.134	0.147	1.2

SB-9	3.708	3.708	0.401	3.4
SW-1	2.693	2.693	0.520	4.5
SW-2	2.805	2.805	0.791	6.8
SW-3	2.784	2.784	0.595	5.1
SW-4	2.883	2.883	0.103	0.9
SW-5	2.489	2.489	0.073	0.6
SW-6	2.359	2.359	0.108	0.9
SW-7	2.973	2.973	0.221	1.9
SW-8	3.134	3.134	0.442	3.8
SW-9	2.566	2.566	0.209	1.8
SD-1	2.770	2.770	0.384	3.3
SD-2	2.614	2.614	0.440	3.8
SD-3	2.745	2.745	0.417	3.6
SD-4	2.565	2.565	0.101	0.8
SD-5	2.378	2.378	0.061	0.5
SD-6	2.238	2.238	0.168	1.4
SD-7	2.874	2.874	0.248	2.1
SD-8	2.932	2.932	0.094	0.8
SD-9	3.450	3.450	0.230	2.0
CB-1	2.792	2.792	0.806	6.9
CB-2	2.615	2.615	0.487	4.2
CB-3	2.748	2.748	0.539	4.6
CB-4	2.956	2.956	0.163	1.4
CB-5	2.936	2.936	0.165	1.4
CB-6	2.533	2.533	0.214	1.8
CB-7	3.708	3.708	0.601	5.1
CB-8	2.069	2.069	0.281	2.4
CB-9	3.516	3.516	0.614	5.3
CW-1	2.770	2.770	0.320	2.7
CW-2	2.796	2.796	0.488	4.2
CW-3	2.770	2.770	0.384	3.2
CW-4	2.232	2.232	0.105	0.9
CW-5	2.977	2.977	0.045	0.4
CW-6	2.327	2.327	0.052	0.4
CW-7	2.990	2.990	0.116	1.0
CW-8	2.695	2.695	0.221	1.9
CW-9	3.186	3.186	0.265	2.4
CD-1	2.551	2.551	0.493	4.2
CD-2	2.740	2.740	0.240	2.0
CD-3	2.777	2.777	0.498	4.3
CD-4	2.549	2.549	0.161	1.3
CD-5	2.729	2.729	0.119	1.0
CD-6	2.160	2.160	0.119	1.0
CD-7	3.129	3.129	0.258	2.2
CD-8	2.223	2.223	0.323	2.8
CD-9	2.950	2.950	0.314	2.7
NB-1	2.614	2.614	0.340	2.9
NB-2	2.766	2.766	0.425	3.7
NB-3	2.717	2.717	0.375	3.2
NB-4	2.037	2.037	0.072	0.6

NB-5	2.272	2.272	0.103	0.9
NB-6	2.958	2.958	0.122	1.0
NB-7	3.342	3.342	0.198	1.7
NB-8	3.510	3.510	0.245	2.1
NB-9	3.640	3.640	0.267	2.3

FIG: 1-10. TOTAL CHLOROPHYLL ESTIMATION CHARTS:

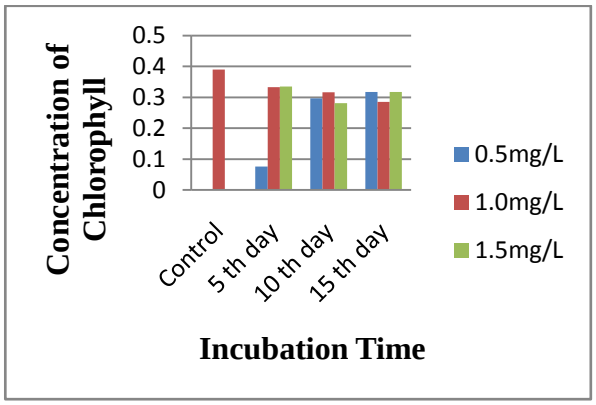


FIG:1. TOTAL CHLOROPHYLL FOR *HALIMEDA GRACILIS* IN BENZENE

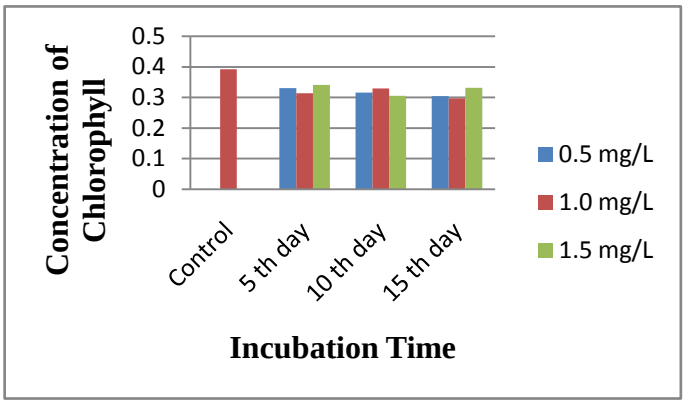


FIG: 2. TOTAL CHLOROPHYLL FOR *HALIMEDA GRACILIS* IN WATER

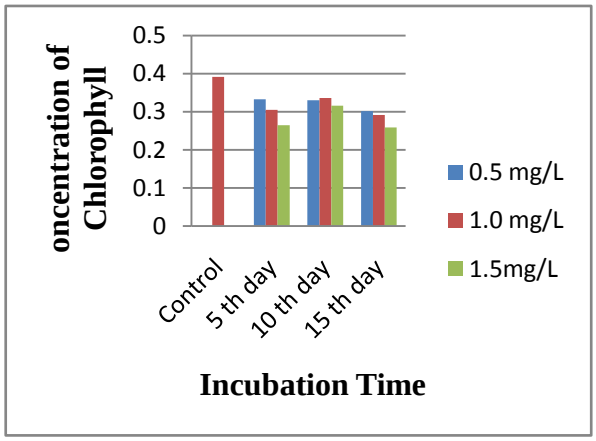


FIG: 3. TOTAL CHLOROPHYLL FOR *HALIMEDA GRACILIS* IN DIETHYL ETHER

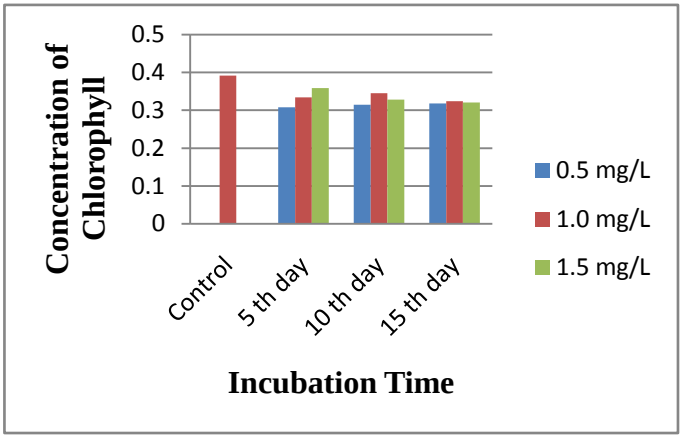


FIG: 4.TOTAL CHLOROPHYLL FOR *CERAMIUM RUBRUM* IN BENZENE

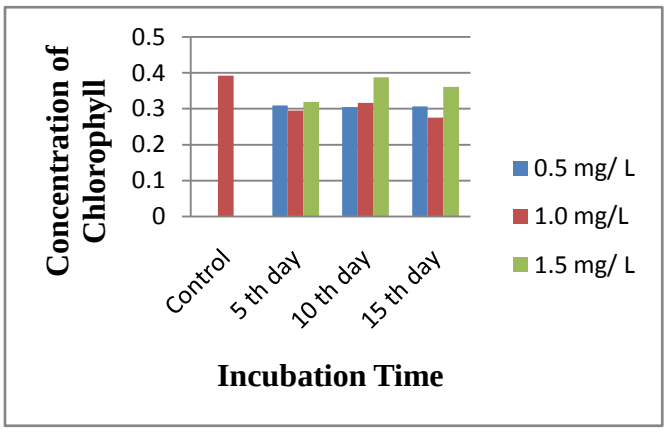


FIG: 5.TOTAL CHLOROPHYLL FOR *CERAMIUM RUBRUM* IN WATER

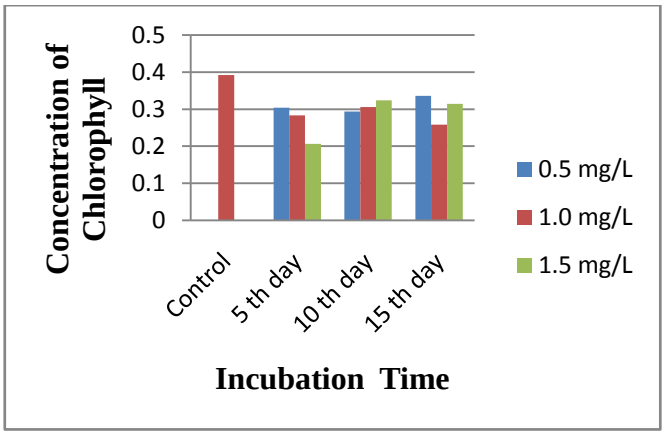


FIG: 6. TOTAL CHLOROPHYLL FOR *CERAMIUM RUBRUM* IN DIETHYL ETHER

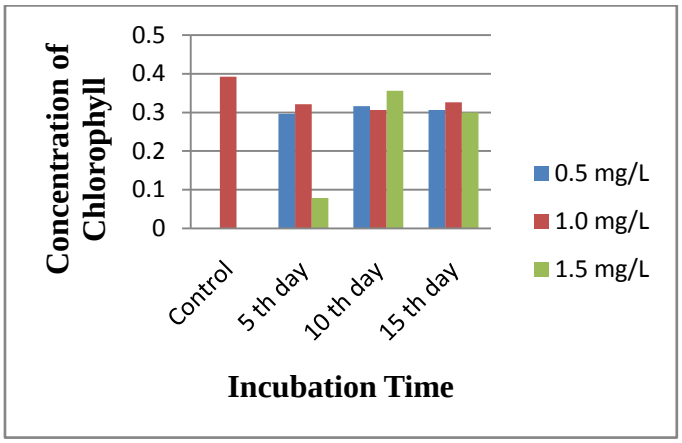


FIG: 7. TOTAL CHLOROPHYLL FOR *CYSTOPHYLLUM MURICATUM* IN BENZENE

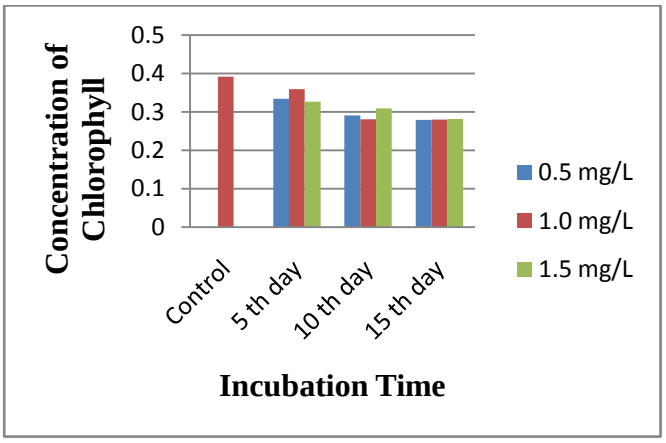


FIG: 8.TOTAL CHLOROPHYLL FOR *CYSTOPHYLLUM MURICATUM* IN WATER

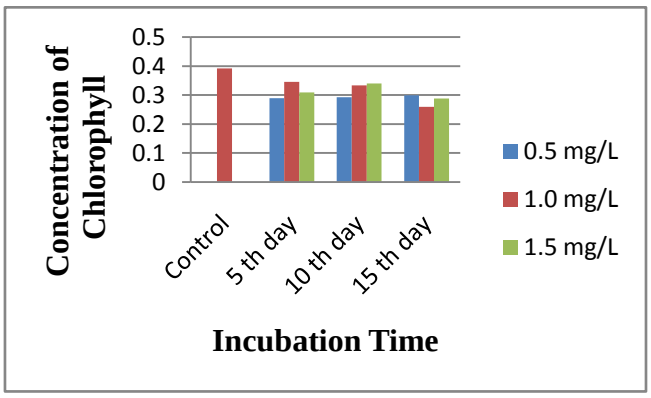


FIG: 9. TOTAL CHLOROPHYLL FOR *CYSTOPHYLLUM MURICATUM* IN DIETHYLETHER

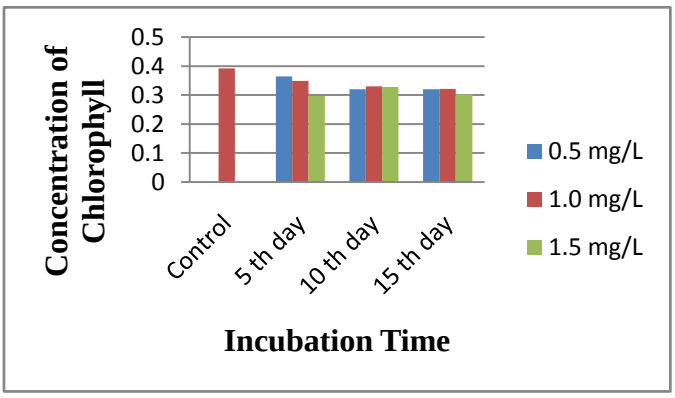


FIG: 10.TOTAL CHLOROPHYLL FOR *NITROBENZEN*

FIG: 11. TOTAL CARBOHYDRATE ESTIMATION CHARTS (FIG. 12-21):

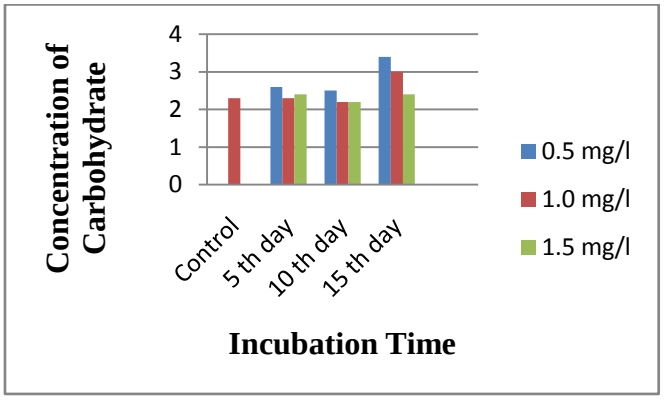


FIG: 12. TOTAL CARBOHYDRATE FOR *HALIMEDA GRACILIS* IN BENZENE

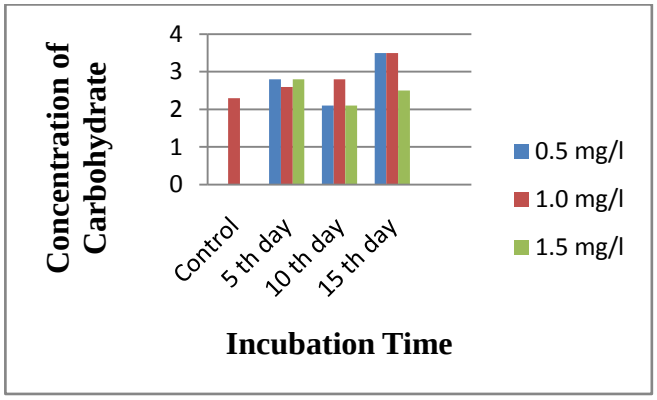


FIG: 13.TOTAL CARBOHYDRATE FOR *HALIMEDA GRACILIS* IN WATER

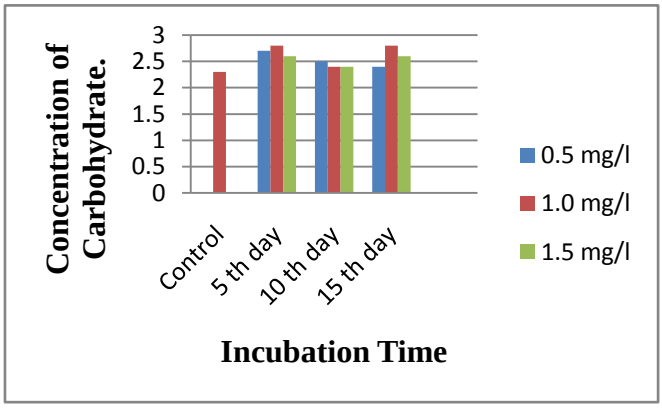


FIG: 14. TOTAL CARBOHYDRATE FOR *HALIMEDA GRACILIS* IN DIETHYEL ETHER

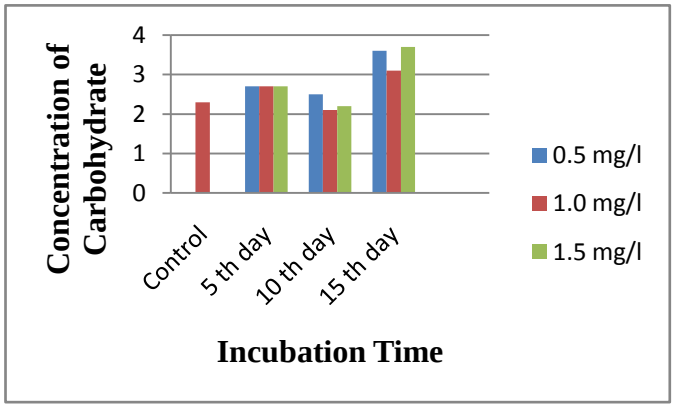


FIG: 15.TOTAL CARBOHYDRATE FOR *CERAMIUM RUBRUM* IN BENZENE

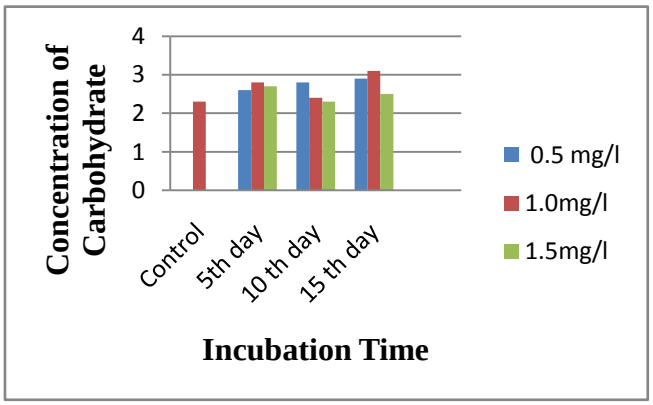


FIG: 16.TOTAL CARBOHYDRATE FOR *CERAMIUM RUBRUM* IN WATER

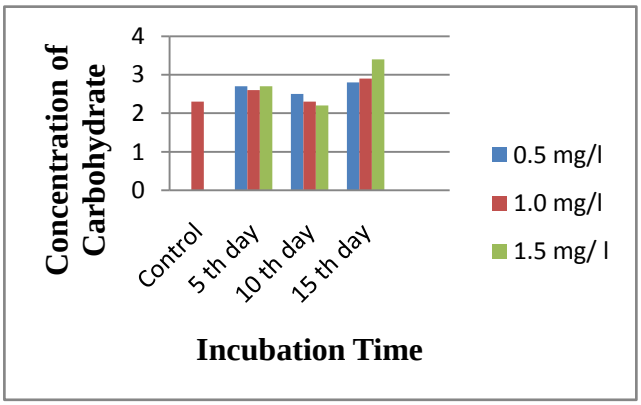


FIG: 17.TOTAL CARBOHYDRATE FOR *CERAMIUM RUBRUM* IN DIETHYEL ETHER

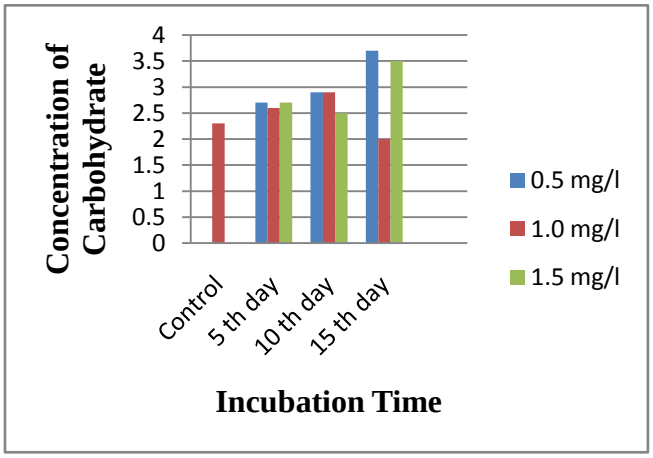


FIG : 18.TOTAL CARBOHYDRATE FOR *CYSTOPHYLLUM MURICATUM* IN BENZENE

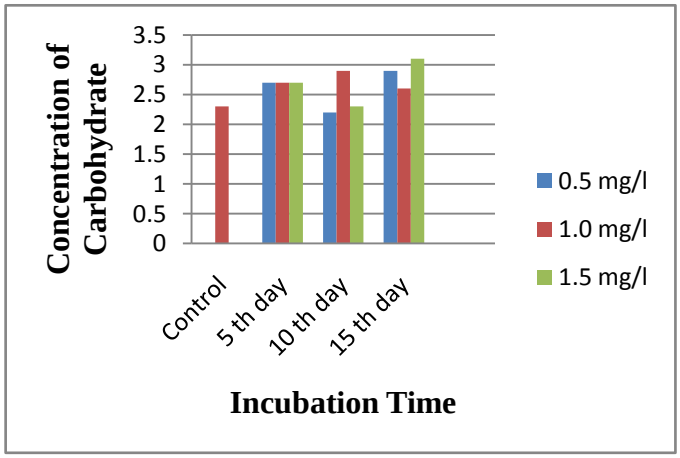


FIG: 19.TOTAL CARBOHYDRATE FOR *CYSTOPHYLLUM MURICATUM* IN WATER

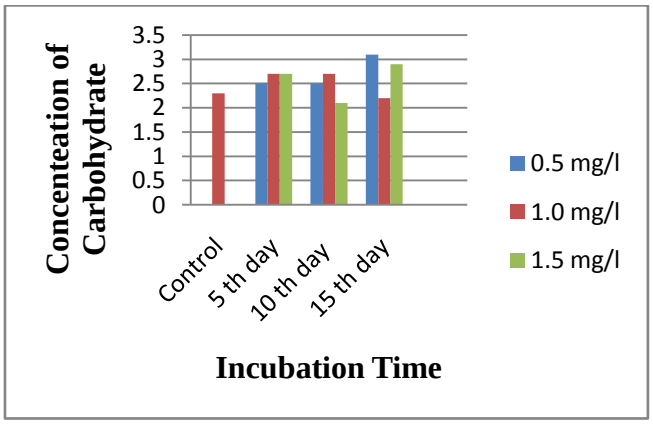


FIG: 20. TOTAL CARBOHYDRATE FOR *CYSTOPHYLLUM MURICATUM* IN DIETHYL ETHER

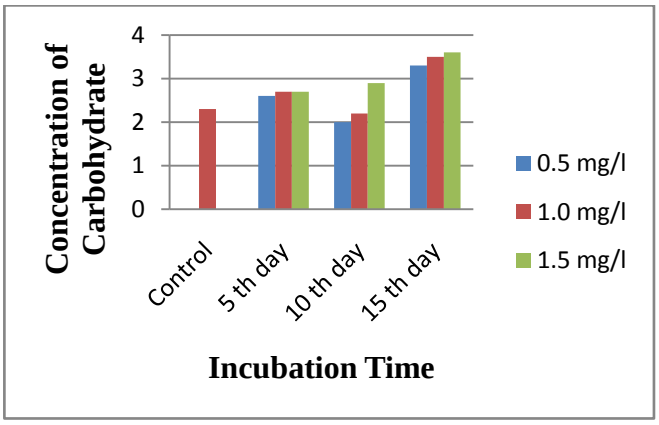


FIG: 21.TOTAL CARBOHYDRATE OF NITROBENZENE

FIG: 22. TOTAL PROTEIN ESTIMATION CHARTS (FIG. 23- 32):

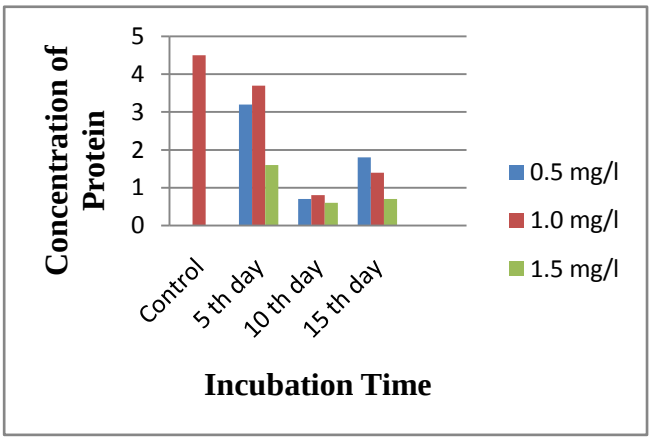


FIG: 23. TOTAL PROTEIN FOR *HALIMIDA GRACILIS* IN BENZENE

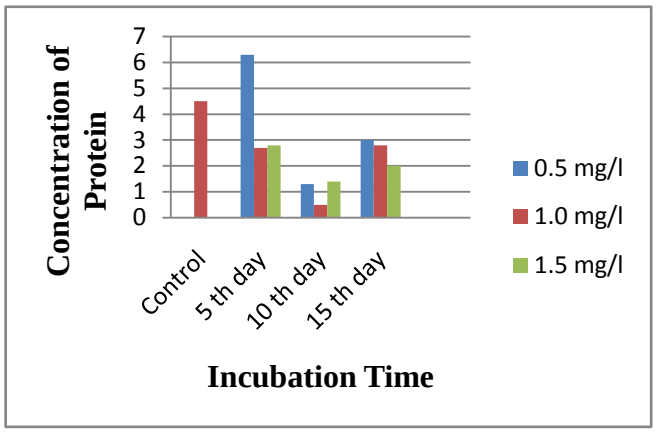


FIG: 24.TOTAL PROTEIN FOR *HALIMIDA GRACILIS* IN WATER

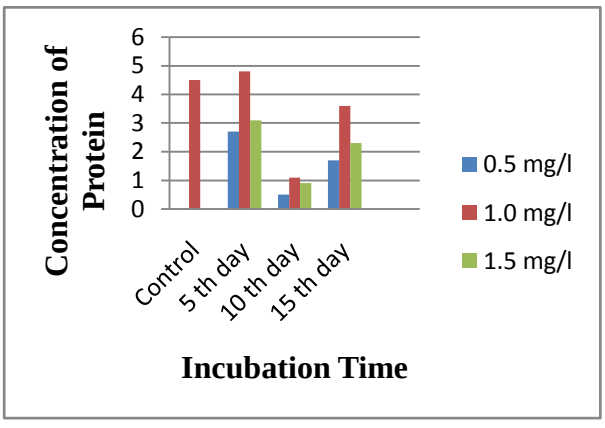


FIG: 25.TOTAL PROTEIN FOR *HALIMIDA GRACILIS* IN DIETHYL ETHER

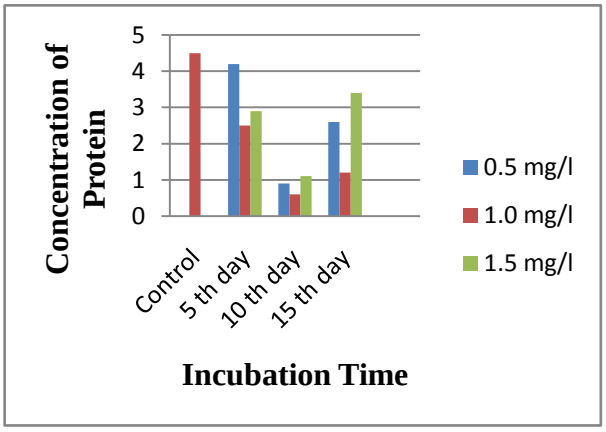


FIG: 26. TOTAL PROTEIN FOR *CERAMIUM RUBRUM* IN BENZENE

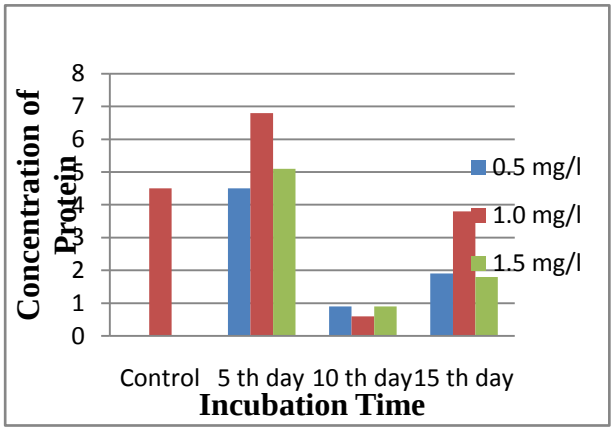


FIG: 27. TOTAL PROTEIN FOR *CERAMIUM RUBRUM* IN WATER

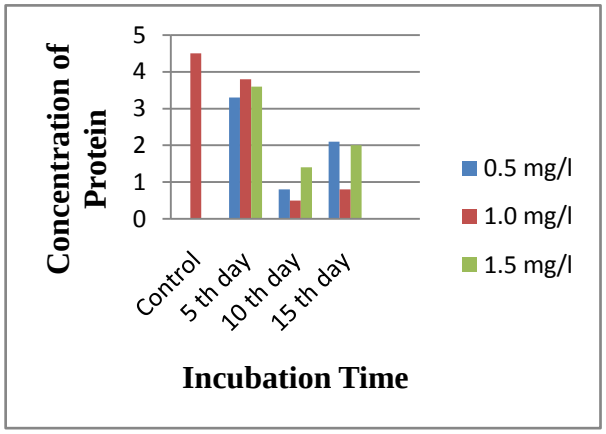


FIG: 28. TOTAL PROTEIN FOR *CERAMIUM RUBRUM* IN DIETHYL ETHER

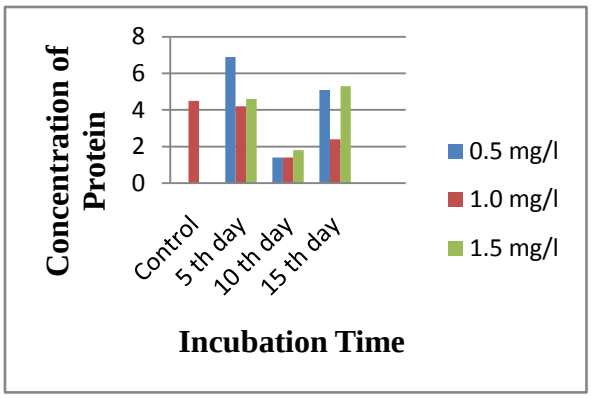


FIG: 29. TOTAL PROTEIN FOR *CYSTOPHYLLUM MURICATUM* IN BENZENE

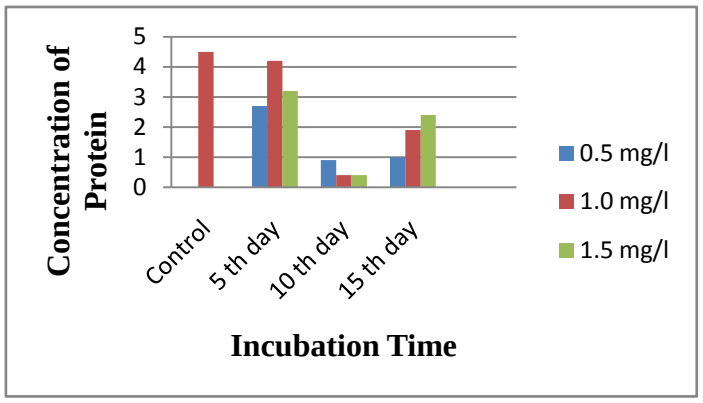


FIG: 30.TOTAL PROTEIN FOR *CYSTOPHYLLUM MURICATUM* IN WATER

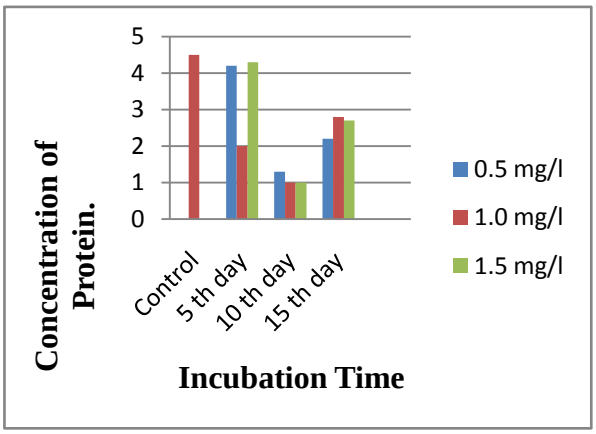


FIG: 31.TOTAL PROTEIN FOR *CYSTOPHYLLUM MURICATUM* IN DIETHYLETHER

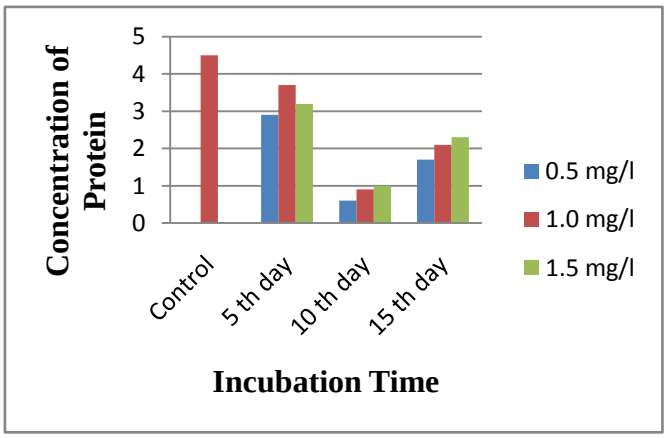


FIG: 32.TOTAL PROTEIN FOR NITROBENZENE

Different extraction methods can be used for seaweed extracts preparation i.e. water extraction under high pressure, alcohol extraction, alkaline extraction, microwave-assisted extraction (MAE) and supercritical CO2 extraction. Conditions of the process depend on the active substances of interest. Extracts rich in auxins can be produced by alkaline extraction. The process is carried out under low pressure. Previously dried probes are extracted with the use of sodium hydroxide (Booth 1996). Extraction in 85% methanol leads to obtainment of algae extract rich in gibberelins. Biomass should be previously homogenized. The temperature of the process is 4°C (Hytonen *et al*., 2009).

Seaweed liquid fertilizer was used for coconut plantation in Tamilnadu and Kerala (Kalimuthu *et al*., 1987). An experimental field trail report of CMFRI (Central Marine Fisheries Research Institute), Mandapam reported that by using 3 months old Hypnea and cowdung compost on Bhendi crop gave 73% higher yield than that of control. Seaweeds are not only used as compost but can also be used as a liquid fertilizer. Liquid seaweed extract when applied to seed, soil or sprayed on crops it increased seed germination percentage, nutrient uptake, growth (Immanuel and Subramanian, 1999).SWC treatment enhanced both root shoot ratios and biomass accumulation in tomato seedlings by stimulating root growth (Crouch and van Staden 1992). Seaweeds and seaweed products enhance plant chlorophyll content (Blunden 1997). Seaweed concentrate trigger early flowering and fruit stein a number of crop plants (Abetz and Young 1983). Tomato seedlings treated with SWC set more flowers earlier than the control plants and this was not considered to be a stress response (Crouch and van Staden 1992). Seaweed extracts have been shown to improve plant resistance against insect and diseases (Allen and others 2001). Our study has helped to extend the period of growth and improved the quality of the plants. Ultimately, the fabricated support presented fertilizer properties, water retention and biodegradability and could serve in horticulture as an alternative to plastic pots and chemical fertilizer (Asma Chbani 2013). The extract of *Enteromorpha intestinalis* enlarged seed germination, root, shoot length and chlorophyll content of *Sesamum indicum* (Gandhiyappan and Perumal 2001). The effect of the liquid extract from *Sargassum wightii* on *Arachis hypogaea* which showed boost in height and quantity of branches of the plant in comparison to chemical treatment (Sridhar and Rengasamy., 2010). The callus from the *Withania somnifera* treated with seaweed extract in vitro in various concentration, the good results were obtain in medium with 40% of seaweed extract, it gives 8.6 shoots / callus. While in higher concentration of seaweed in medium ie: 80% produce 4.3 shoots / callus (Sathees kannan.,2014).The *Arachis hypogea* L. seeds were treated with seaweed extract in various concentration and the fantastic result were obtain in 2%. The fresh weight, dry weight, root, shoot length, number of branches, leaf, protein, lipid, chlorophyll, carbohydrate were obtain high while compare to others. The leaf tissue of 2% SLF treated groundnut sample and control plant leaf tissue were analysis in scanning electron microscopic with energy Dispersive spectroscopic analysis the elements varies (Ganapathy selvam and Sivakumar 2014).

Seaweed *Caulerpa racemosa* extract 3% spray at vegetative stage and flowering stage increase in chlorophyll, crop growth rate, Seed weights, while compare to control (Sujatha and Vijayalakshmi 2013). The GC-MS analysis of *Caulerpa racemosa* extract shows much of plant nutrients, trace elements and antioxidant. The foliar spray of *Kappaphycus alvarezii* 5% enhances the fruit and the foliar spray plant leaves shown antibacterial activity and resistance to fruit borers compare to control (Zodape *et al.,* 2011).

According to Jeyakumar, Department of Crop biology Tamilnadu Agricultural University, Coimbatore, Nitrobenzene is a Combination of nitrogen and plant growth regulators, extracted from seaweeds. Nitrobenzene produces best results in combination of plant growth regulators, which have capacity to increase flowering in plant and also prevent flower shedding. Yield contributing characters like plant height increase by 8-10% and number of branches per plant increase by 15-20%. Four sprays of Nitrobenzene during 40,55,80 and 105 DAS improve the yield up to 40%.

Groundnut is a legume of economic importance, its improvement could greatly benefit from the integration of both classical and modern techniques. Although biotechnology techniques such as tissue culture and gene transformation have been reported in peanut. The different concentration of 2,4-D induced callus. The 2 mg/L 2,4-D gives maximum level of Callus (Alamand Khaleque 2010). The callus from hypocotyls explants were obtained in 0.5 mg/ L of 2,4-D long with BAP (Muthusamy *et al*., 2007). Maximum level of callus from mature tissues were obtained from 3.0 mg/ L of IAA and 1.0 mg/L of BAP (Palanivel *et al*., 2002).

IV. CONCLUSION

In present study totally three seaweeds namely *Halimeda gracilus, Ceramium rubrum* and *Cystophyllum muricatum* were used. The seaweeds were extracted with three different organic solvents such as benzene, diethyl ether and water. Totally nine extracts were obtained from three seaweeds. Among the nine extracts, *Cystophyllum muricatum* aqueous extract gave

high amount of crude extract 3.150grams, Callus Bioassay was done at different concentration (0.5,1.0,1.5 mg L^{-1}) at different incubation (5, 10, 15 days). *Ceramium rubrum* benzene extract showed increased level of total carbohydrate content, while the total protein level increased in *Cystophyllum muricatum* benzene extract and there was no increase in total chlorophyll content with all the extracts tested. This is the first report on application of seaweed extract and nitrobenzene granule, a flower enhancing hormone in *A.hypogaea* callus culture. GC – MS and LC-MS have to be done for further characterization of the bio molecules present in the three seaweed different solvent, water extracts and TLC for confirmation of Nitrobenzene compound.

ACKNOWLEDGEMENTS

We would be thankful to M.B.Viswanathan, Head, Department of Botany, Bharathidasan University, Trichy-24 for providing the Lab facilities to carry out the research.

REFERENCES

[1] Abetz, P, Young, CL (1983) The effect of seaweed extract sprays derived from Ascophyllum nodosum on lettuce and cauliflower crops. Botanica Marina 26: 487-492.

[2] Alam A K M and Khaleque M A. 2010. In vitro response of different explants on callus development and plan t regeneration in groundnut. lnt. J. Expt. Agric 1(1) 1-4.

[3] Asma Chbani, Hiba Mawlawi and Laurence Zaouk (2013) Evaluation of brown seaweed (*Padina pavonica*) as biostimulant of plant growth and development African Journal of Agricultural Research 8(13), 1155-1165.

[4] Asma Chbani, Hiba Mawlawi and Laurence Zaouk (2013)Evaluation of brown seaweed (*Padina pavonica*) as biostimulant of plant growth and development African Journal of Agricultural Research 8(13), 1155-1165.

[5] Blunden G, Jenkins T, Liu Y (1997) Enhanced leaf chlorophyll levels in plants treated with seaweed extract. J Appl Phycol 8:535–543

[6] Booth B.: The manufacture and properties of liquid seaweed extracts, Proceedings International Seaweed Symposium, 1969, 6, 655-662.

[7] Brain KR, Chalopin MC, Turner TD, Blunden G, Wildgoose PB (1973) Cytokinin activity of commercial aqueous seaweed extract. Plant Sci Lett 1:241–245

[8] Crouch IJ, van Staden J (1992) Effect of seaweed concentrate on the establishment and yield of greenhouse tomato plants. J Appl Phycol 4:291–296

[9] Crouch IJ, van Staden J (1993a) Evidence for the presence of plant growth regulators in commercial seaweed products. Plant Growth Regul 13:21–29

[10] Donata Dubber, Tilmann Harder Extracts of Ceramium rubrum, Mastocarpus stellatus and Laminaria digitata inhibit growth of marine and fish pathogenic bacteria at ecologically realistic concentrations. Science Direct 2007, Aquaculture 274 (2008) 196–200.

[11] Durand N, Briand X, Meyer C (2003) The effect of marine bioactive substances (NPRO) and exogenous cytokinins on nitrate reductase activity in Arabidopsis thaliana. Physiol Plant 119:489–493

[12] Ganapathy selvam and Sivakumar , 2014 Influence of seaweed extract as an organic fertilizer on the growth and yield of Arachis hypogea L. and their elemental composition using SEM– Energy Dispersive Spectroscopic analysis Asian Pacific Journal of Reproduction 10.1016/S2305-0500(13)60179-7.

[13] Gandhiyappan, K. and Perumal, P. 2001. Growth promoting effect of seaweed liquid fertilizer (Enteromorpha intestinalis) on the sesame crop plant (Sesamum indicum L.). Seaweed Res. Utiln. 23: 23-25.

[14] Hussain A, Boney AD (1973) Hydrophilic growth inhibitors from Laminaria and Ascophyllum. New Phytol 72:403–410

[15] Hytönen T., Elomaa P., Moritz T., Junttila O.: Gibberellin mediates daylength-controlled differentiation of vegetative meristems in strawberry (Fragaria x ananassa Duch), BMC Plant Biology 2009, 9(18), 1471-2229.

[16] Immanuel, R. and Subramanian, S. K. 1999: Effect of fresh extract and seaweed liquid fertilizers on some cereals and millets. Seaweed Res. Utiln. 21: 91-94.

[17] International Journal of Advancements in Research & Technology, Volume 2, Issue 10, 216 ISSN 2278-7763.

[18] Kalimuthu, S., Chennubhotla, V. S. K. and Kaliaperumal, N. 1987. Economic important seaweeds of Inida. Seaweed Research and Utilization in India. CMFRI Bulletin. 41: 99.

[19] Kamalesh Prasad,Arun Kumar Das, Mihir Deepak Oza, Harshad Brahmbhatt, Arup Kumar Siddhanta, Ramavatar Meena, Karuppanan Eswaran, Mahesh Rameshchandra Rajyaguru, and Pushpito Kumar Ghosh 2010 Detection and Quantification of Some Plant Growth Regulators in a Seaweed-Based Foliar Spray Employing a Mass Spectrometric Technique sans Chromatographic Separation J. Agric. Food Chem. 2010, 58, 4594–4601

[20] Lowry, OH, NJ Rosbrough, AL Farr, and RJ Randall. *J. Biol. Chem. 193:* 265. 1951.

[21] Matysiak K., Kaczmarek S., Kierzek R., Kardasz P.: Ocena działania ekstraktów z alg morskich oraz mieszaniny kwasów huminowych i fulwowych na kiełkowanie i początkowy wzrost rzepaku ozimego (Brassica napus L.), Journal of Research and Applications in Agricultural Engineering, 2010, 55(4), 28-32.

[22] Muthusamy .A., K. Vasanth, D. Sivasankari, B. R. Chandrasekar, N. Jayabalan 2007,Effects of mutagens on somatic embryogenesis and plant regeneration in groundnut Biologia Plantarum September 2007, Volume 51, Issue 3,430-435.

[23] Ordog V, Stirk WA, van Staden J, Novak O, Strnad M (2004) Endogenous cytokinins in the three genera of microalgae from the Chlorophyta. J Phycol 40:88–95.

[24] Palanivel S, S. Parvathi and N.Jayabalan 2002 Callus induction and plantlet regeneration for mature cotyledon segment of groundnut (Arachis hypogaea. L). J.Plant,Biol.45,22-29.

[25] Reitz SR, Trumble JT (1996) Effects of cytokinin-containing seaweed extract on *Phaseolus lunatus L*.: influence of nutrient availability and apex removal. Bot Mar 39:33–38

[26] S. M. Hassan* and H. R. Ghareib 2009 Bioactivity of *Ulva lactuca L*. acetone extract on germination and growth of lettuce and tomato plants.African Journal of Biotechnology

[27] Spinelli, F., Fiori, G., Noferini, M., Sprocatti, M. and Costa, G.. Perspectives on the use of a seaweed extract to moderate the negative effects of alternate bearing in apple and strawberry. J. Horticut. Sci. Biotnol 2010. Special issue: 131-137.

[28] Spinelli, F., Fiori, G., Noferini, M., Sprocatti, M. and Costa, G. 2010. Perspectives on the use of a seaweed extract to moderate the negative effects of alternate bearing in apple and strawberry. J. Horticut. Sci. Biotnol. Special issue: 131-137.

[29] Sridhar, S. and Ramasamy R. 2010 b. Significance of seaweed liquid fertilizer for minimizing chemical fertilizers and improving yield of Arachis hypogaea under field trial. Recent Res. Sci. Tech. 2: 73-80.

[30] Stirk W.A., Staden J., Novák O., Hradecká V., Pencik A., Rolcik J., Stranad M., Van Staden J.: Endogenous cytokinins, auxins and abscisic acid in Ulva fasciata (Chlorophyta) and Dictyota humifusa (Phaeophyta): towards understanding their biosynthesis and homoeostasis, European Journal of Phycology, (2009), 44(2), 231-240.8(16), . 3832-3838

[31] Sujatha and Vijayalakshmi .,2013 Foliar application of *Caulerpa racemosa* seaweed extract as bio-stimulant for enhancement of growth and yield of blackgram (*Vigna mungo* L.)

[32] Tay SA, Macleod JK, Palni LM, Letham DS (1985) Detection of cytokinins in a seaweed extract. Phytochemistry 24:2611–2614

[33] Wajahatullah Khan, Usha P. Rayirath, Sowmyalakshmi Subramanian, Mundaya N. Jithesh,Prasanth Rayorath, D. Mark Hodges, Alan T. Critchley, James S. Craigie, Jeff Norrie, Balakrishan Prithiviraj.Seaweed Extracts as Biostimulants of Plant Growth and Development, Journal of plant growth regulator, 2009, 28 (4) 386-399.

[34] Yokoya N.S., Stirk W.A., Staden J., Novák O., Turecková V., Pencik A., Strnad M.: Endogenous cytokinins, auxins, and abscisus acid in red algae from Brazil, Journal of Phycology, 2010, 46(6), 1198?-1205.

[35] Zodape, S. T., Gupta, A. and Bhandari, S. C. 2011. Foliar application of seaweed sap as biostimulant for enhancement of yield and quality of tomato. J. Sc. Ind. Res. 67: 215-219.

[36] Yurima Cortés, Emilio Hormazábal, Hellmuth Leal, Alejandro Urzúa, Ana Mutis.Leonardo Parra, Andrés Quiroz. Novel antimicrobial activity of a dichloromethane extract obtained from red seaweed *Ceramium rubrum* (Hudson) (Rhodophyta: Florideophyceae) against *Yersinia ruckeri* and *Saprolegnia parasitica*, agents that cause diseases in salmonids.Electronic Journal of Biotechnology2014, Volume 17, Issue 3 126–131.

Process Optimization, Consumer testing and Shelf-life Determination of Canned "*Halang-halang*": A Filipino Traditional Food

Pet Anthony L. Pascual[1], Gladys L. Pascual[2]

[1]Leyte Normal University (LNU), Department of Home Arts Entrepreneurship, Tacloban City, Philippines
[2]Southern Leyte State University (SLSU)-Bontoc Campus, Bontoc, Southern Leyte, Philippine

Abstract— *Halang-halang and tagutoare the Filipino names given to the hot and spicy pulutan (finger food) prepared from finely chopped across of young native chickens stewed in coconut milk with chopped chillies and spices. The basic formulation and process of halang-halang need to be assessed especially as canned product for longer shelf life and wider distribution. Thus, this research optimizes the formulation, processing conditions and determines shelf-life and proximate composition of canned halang-halang. Three identified factors were used in the optimization experiment following the Box-Benhken Design (BBD)with 15 treatments. Three levels of coconut milk (0, 20, 40%), sautéing time (0, 3, 6 minutes) and processing time (30, 40 and 50 minutes) were used. Different treatments were subjected to sensory evaluation, optimization and verification test. Response surface regression (RSREG) analysis was used to determine the optimum level of coconut milk, sautéing and processing time combinations. Optimum formulation was achieved at mid-level coconut milk (20%), longer (50 minutes) or shorter (30 minutes) processing time and at any sautéing time. Both coconut milk level and processing time significantly influenced the sensory qualities of the product, while sautéing time showed no significant effect in all response variables. Verification test confirms that optimum formulation is better in acceptability scores compared to treatment outside the optimum region. Both the developed "plain" and "hot" halang-halang formulations have high preference from young and adult consumers. After 15 months of storage, canned halang-halang products were still microbiologically acceptable showing a microbial count much lower than the safe level.*

Keywords— Traditional food, Box-Benhken Design, canning, proximate analysis, shelf-life determination.

I. INTRODUCTION

Poultry has become increasingly popular in recent years, because it is inexpensive, versatile and considered a cheaper source of protein. The ability of poultry to adapt to most areas of the world, the rapid growth rate of poultry and the rapid generation time, all make poultry an ideal rich source of animal nutrients for human food. It has been estimated that chicken appears in the diet of more people throughout the world as a source of meat than the meat of any other animal (Labensky and Hause, 1995).According to Buzby and Farah (2006), the increase in consumption of chicken has doubled between 1970 and 2004, from 27.4 pounds per person to 59.2 pounds. Chicken consumption has been gaining ground against beef.

The Philippine native chicken (*Gallus gallusdomesticus*) is a great substitute for commercial poultry chicken. Generally known to be self-supporting and well adapted to their scavenging existence, they survive and reproduce under minimal care and management. Many consumers feel that chickens that are allowed to move freely and forage for food have a better flavor compared to chickens that are raised indoors in chicken houses. Moreover, certain flavor characteristics of their meat and eggs have fancily induced some people to continue raising these indigenous and nondescript mongrels over that of the commercial hybrid broiler.

There has been an increasing demand around the world for spices and spicy foods during the last few years because of the increased production of convenience foods. Spices improve the flavor and give pungency to foods. Some spice components also are known to act as microbial antagonists (Lai and Roy, 2004). The meat industry and soup manufacturers use the largest quantities of spices.

Halang-halang and Taguto are the Filipino names given to this hot and spicy food usually consumed as *pulutan* (finger food) for most beer drinkers in the province of Leyte, Philippines. The cooking of *halang-halang* came from the idea of utilizing the young female chickens that are culled or considered useless for fighting cock purposes. It is made from finely chopped carcass of young (approximately 4 months old) mostly female native chickens stewed in coconut milk with high amount of chopped chili and spices. Since the bones are included it is rich in major nutrients like calcium. *Halang-halang* is also

considered as an ethnic product that actually originated in Leyte. This product might be consumed as viand if it is with plain or unspiced formulation.

Halang-halang is not new since many people living in the southern villages in Leyte, Philippines have already tasted this product (J.M.Lasquites, personal communication, January 20, 2014). However, its basic formulation and process need to be optimized especially as a canned product for longer shelf life and wider distribution. This could make it possible for this poultry product to be transported to different areas throughout the world. Thus, this research project was conducted to optimize the formulation, processing conditions, consumer testing and shelf-life determination of canned *halang-halang*.

Specific Objectives of the Study

1. Determine the consumer acceptability of canned plain "*halang-halang"* at different parameter levels and combinations.
2. Determine the optimum combination of coconut milk, length of sautéing and retort time for canned *halang-halang* based on consumer acceptability.
3. Determine the consumer preference of the "plain" and "hot" *halang-halang* samples.

II. MATERIALS AND METHODS

2.1 Experimental Design

Following the Box-Benhken Design (Meyer and Montgomery, 1995) a 3^3 fractional factorial design was followed using coconut milk, length of sautéing and retort time combinations as independent variables. A total of 15 treatments (Table 1) were used including three replications at the center point. Chosen levels are 0, 20 and 40% coconut milk: 0, 3, and 6 min sautéing time and 30, 40, and 50 minutes for retort time.

TABLE 1
THE DIFFERENT TREATMENTS OF THE BOX-BEHNKEN DESIGN FOR 33 FRACTIONAL FACTORIAL EXPERIMENTS AS APPLIED IN EVALUATING THE QUALITY OF CANNED "PLAIN" *HALANG-HALANG*.

Treatment	Sautéing time (minutes)	Coconut milk (%)	Retort time (minutes)
1	0	20	30
2	6	20	30
3	6	20	50
4	0	20	50
5	0	40	40
6	3	40	30
7	6	40	40
8	3	40	50
9	0	0	40
10	3	0	30
11	6	0	40
12	3	0	50
13	3	20	40
14	3	20	40
15	3	20	40

At 100% chicken

2.2 (a) Preparation of Ingredients

Chicken Meat. The cleaned carcass was cut into quarters and chopped into small pieces of about 0.3 to 0.4 cm in length.

Coconut milk. Matured coconuts were dehusked, grated and milk extracted without added water. The extracted milk was divided into two equal portions and was placed in separate containers.

Other Ingredients. Chilies, onion bulb and onion stalks were chopped into small pieces before use.

2.2 (b) Preparation of Canned *Halang-halang*

***Cooking*.** Different spices such as ginger (62.96g), and garlic (37g) together with the chopped poultry (1000g) were sautéed in a frying pan until light brown. Half portion of the coconut milk (150g) was added to the mixture and was stirred occasionally until boiling. Monosodium glutamate (3.7g), salt (55g) and chopped chillies (18g) for "hot" formulation were added and were cooked for 5 minutes. Onions (74g) and the remaining portion of the coconut milk (150g) was the added to the final mixture and cooked for 3 minutes.

Cleaning of Cans. Container cans were cleaned and sterilized.

Filling. The filling of the cooked product was done manually right after cooking into sterile cans.

Seaming. The filled cans were covered with can cover and were sealed using a double seam can sealer. Double seam sealing was employed in closing the cans. Careful attention was done and possible leakage and distorted seams were checked for after seaming.

Processing. The sealed cans were placed in an autoclave. The autoclave was tightly closed and was switched on. The stopcock of the autoclave was opened first and was closed 3 minutes after the steam came out from the cock. Counting of retort time started when the retort temperature has already reached 250^{o}F. Different retort times were used at a constant retort temperature of 250^{o}F.

Cooling. After retorting, the autoclave was switched off and the stopcock was slightly opened. The autoclave was allowed to register a zero pressure reading before it was opened. The hot cans were removed and were rinsed in cold water to facilitate the cooling process. The cans were wiped with clean, dry cloth and were stored in a cool dry place.

***Can Seam Evaluation*.** External check-up of can seam was done to check for possible gross abnormalities. Checking and measuring of seamed can samples were done carefully along the periphery of the can seam with the aid of a micrometer calliper.

2.3 Sensory Evaluation

All sensory evaluation activities were carried out to determine the acceptability of the product ("plain" formulation) from the 15 different treatments right after a day of storage. Samples from the different treatments were presented using an Incomplete Block Design to a group of 28 panelists composing of students who evaluated the products' color, aroma, texture, flavor, and general acceptability.

2.4 Optimization

Response surface regression (RSREG) analyses using SAS Statistical Computer Software (SAS 9.1.3 Portable)to determine the effect of the independent variables on the sensory qualities of the product and to determine the optimum coconut milk, sautéing and retort time combinations was conducted. The samples for analysis were selected through randomized collection. Response surface plots were made for all analyses as reference points using STATISTICA computer program to clarify the different effects of factor variables on the responses studied.

2.5 Verification

Verification tests were conducted in duplicate using two selected treatments. Twenty-eight (28) panelists were randomly selected from the 60 that participated during the sensory acceptance test. The treatments included one within and one outside the optimum zone. A paired t-test was done to determine if the actual values was different from the predicted.

2.6 Consumer Preference Test

The treatment in the verification test that was within the optimum zone was used in the consumer preference test and was referred as the "plain" sample. The other treatment was of the same formulation but with added chopped chili (18g) and was referred as the "hot" sample. The consumers evaluated the acceptability of the products and their preference between the two treatments was determined. One hundred consumer panelists were employed consisting of adult consumers. The consumer preference test was done on the samples after 1 week of storage.

2.7 Microbial Analysis

Plate Count Agar (PCA) was used as the medium for the determination of total plate count of microorganisms on the canned *halang-halang* after 0, 3, 6, 9, 12 and 15 months of storage at room temperature. It was conducted on the "hot" *halang-halang* samples. Buffered dilution water was used as dilution blank in the preparation of serial dilution standard plating.

Standard plating procedure was followed for microbial load determination of the samples. Duplicate plates per dilution were made in order to obtain more reliable results. The plates were incubated for 3-5 days at 27ºC. Microbial growth was counted and was reported as colony forming units (CFU) per gram sample.

III. RESULTS AND DISCUSSION

3.1 Sensory Quality Characteristics of Canned *Halang-halang*

Sensory quality characteristics of canned *halang-halang* namely, color, aroma, texture, flavor and general acceptability were affected by the sautéing time, levels of coconut milk as well as the retort time used during processing.

3.1.1 Color Acceptability

The color acceptability values ranged from 5.071 to 7.571 with an overall response mean of 6.735. The conditions predicted for optimum color acceptability of 6.412 were: –6.378 minutes sautéing time, 32.910% coconut milk level, and 35.264 minutes retort time. The predicted optimum condition for sautéing time was located outside the region which was relatively smaller than the studied time; however predicted conditions for coconut milk and retort time were at higher and lower level respectively. This shows that higher coconut milk level, shorter retort time and 0 minutes sautéing time were more favorable for higher color acceptability. This might be true since higher combinations of sautéing time and retort time would destroy proteins and caramelizes sugar present in the coconut milk producing a darker brown color. The predicted maximum sensory score for color acceptability falls between *like slightly* to *like moderately*, implying that canned *halang-halang* is relatively acceptable to the consumer panel.

3.1.2 Aroma Acceptability

The aroma acceptability values for the different canned *halang-halang* samples *which* ranged from 5.821 to 7.535 had an overall response surface mean of 6.892. Statistical analysis (data not shown), revealed that the response on aroma was only affected by coconut milk level and its quadratic effects. This indicates the dependence of aroma acceptability on coconut milk level and its independence on the other variables. The conditions predicted for optimum aroma acceptability of 7.076 of the product were: 3.159 minutes sautéing time, 31.310% coconut milk level and 42.946 minutes retort time.

3.1.3 Texture Acceptability

The texture acceptability which ranged from 5.357 to 7.464 had an overall response mean of 6.623. The conditions predicted for optimum texture acceptability of 6.543 of the canned *halang-halang* were: 2.637 minutes sautéing time, 29.408% coconut milk level, and 38.455 minutes retort time. This implies that shorter sautéing time combined with mid-level of coconut milk and shorter retort time would yield higher texture acceptability scores. Such combinations may result in a product with tougher texture. The preference of tougher texture may be due to the toughness of commercially available canned chicken product and could have influenced the texture acceptability evaluation.

3.1.4 Flavor Acceptability

The flavor acceptability values of canned *halang-halang* samples ranged from 4.785 to 7.678 with an overall response mean of 6.697. Statistical analysis (data not shown) indicates that flavor response was only affected by coconut milk level and its' quadratic effects. This implies that flavor acceptability of canned *halang-halang* depends mainly on the coconut milk level. The predicted optimum condition for flavor acceptability of canned *halang-halang* was 7.06. The predicted response occurred at an optimum condition of -0.019 minutes sautéing time, 42.39% coconut milk level and 35.93 minutes retort time. The predicted optimum condition of sautéing time and coconut milk level was located outside the region which was respectively smaller and larger than the studied level; however predicted condition for retort time was at a lower level. This implies that higher flavor acceptability is achieved at 0 minute sautéing time with the highest level of coconut milk combined with lower retort time. According to Fox and McSweeney (2006), higher temperature during sterilization yields to the production of acrid flavor (hydroxymethyl furfural).

3.1.5 General Acceptability

The general acceptability of canned *halang-halang* samples ranged from 5.321 to 7.821 and had an overall response mean of 6.885. The conditions predicted for optimum general acceptability of 7.00 of the canned samples were: 4.79 minutes sautéing time, 27.63% coconut milk level and 39.55 minutes retort time. This indicated that mid level of sautéing time, coconut milk and retorting time obtained the optimum general acceptability. The predicted maximum general acceptability score falls within *like moderately* in the Hedonic scale used in the study.

3.2 Attaining the Optimum Formulation

The contour plots (Figures 1a, 1b, and 1c) represent an idea as to which combinations of sautéing time, coconut milk level and retort time could result in a product with certain acceptability level. The shaded regions represent values for consumer acceptance for a particular attribute of canned halang-halang corresponding to scores of $\geq$ 6.50, which lie within like slightly to like moderately in the 9-Point Hedonic Scale. The contour plots when overlapped showed that consumer acceptance for texture seemed to be the limiting factor during the optimization procedure. A large area satisfied the requirement of score $\geq$ 6.50. The optimum region required a combination of higher or lower retort time of approximately 50 or 30 minutes, a mid-level coconut milk (20%) at any sautéing time (0 to 6 minutes). Four treatments were within the given optimum region. The treatments from the Box-Behnken design with 0 and 6 minutes sautéing time at 20% coconut milk and 30 minutes retort time and treatments with 6 and 0 minutes sautéing time at 20% coconut milk and 50 minutes retort time from the (T_1, T_2, T_3 and T_4 respectively) satisfied the optimum region.

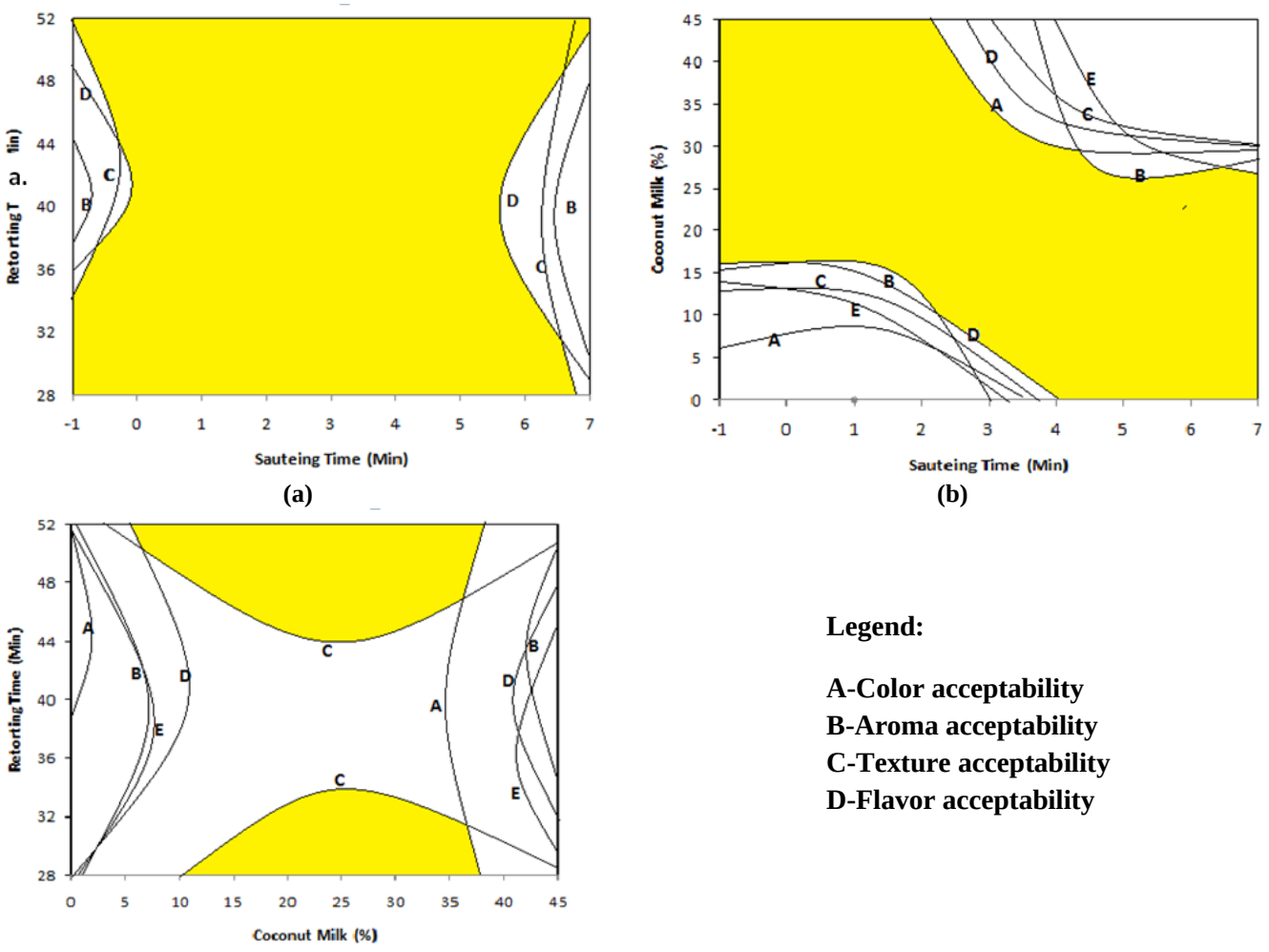


FIGURE 1: a-c. Overlapped contour plots of color, aroma, texture, flavor, and general acceptability of canned *halang-halang* as affected by the combinations of: (a) retort time and sautéing time at constant coconut milk (20%), (b) coconut milk level and sautéing time at constant retort time (50 minutes), and retort time and coconut milk level at constant sautéing time (3minutes).

3.3 Verification of the Optimized Region

Verification tests revealed the predictive ability of all models developed as shown by non-significant differences between observed and predicted values of the treatments tested at 5 % level of significance (Table 2) for all sensory qualities even that of the treatment outside the optimum region. This implies that the models used fit the regression.

TABLE 2
MEAN VALUES OF THE SENSORY QUALITIES OF THE PREDICTED AND ACTUAL OPTIMUM FORMULATION OF CANNED *HALANG-HALANG*.

Parameter	Treatment X		Treatment Y	
	Predicted	Actual	Predicted	Actual
Color	7.39 ± 0.43	7.75 ± 0.42	6.53 ± 0.57	6.21 ± 0.38
Aroma	7.39 ± 0.21	7.67 ± 0.20	7.17 ± 0.43	6.67 ± 0.39
Texture	7.46 ± 0.32	7.21 ± 0.35	6.89 ± 0.47	6.39 ± 0.42
Flavor	7.35 ± 0.38	7.46 ± 0.47	7.67 ± 0.25	7.28 ± 0.28
General Acceptability	7.25 ± 0.50	7.67 ± 0.35	7.07 ± 0.42	6.71 ± 0.23

n =28 panelists
X = treatment inside the optimum region (6 minutes sautéing time; 20% coconut milk and 50 minutes retorting time)
Y = treatment outside the optimum region (6 minutes sautéing time; 40% coconut milk and 40 minutes retorting time)

3.4 Consumer Preference Test

Canned "hot" and "plain" *halang-halang* samples were subjected to consumer preference test. The consumer preference panel was composed of mostly male respondents (85%) since they would likely be the target consumers. Sixty percent (60%) of the respondents were students and the remaining 40% were non-students.

For the "plain" *halang-halang*, 24% of the respondents extremely liked the product, 56% moderately liking the product, while only 10% disliked the product. On the other hand, for the "hot" *halang-halang*, 52% of the respondents extremely liked the product, 34% moderately liked the product, while only 9% disliked the product. This implies that the two canned *halang-halang* products were very acceptable to the consumers.

A large number of the respondents, however, preferred one product from the other. 68% of the respondents preferred the "hot" canned *halang-halang* than the plain one (32%).

3.5 Microbial Analysis

TABLE 3
STANDARD PLATE COUNTS (CFU/G) OF CANNED "HOT" *HALANG-HALANG* AT DIFFERENT STORAGE PERIODS.

	Storage Periods (Mos.)					
	0	3	6	9	12	15
Microbial counts	<10	<10	<10	<10	<10	<10

Results in Table3 show that all microbial counts from canned *halang-halang* at storage periods 0 to 15 months are all less than 10CFU/g. The number of microorganisms is below the tolerable level. Based on the specification from the center for Food Safety (2014), the safe level for microbial counts of canned products immediately after removal from container is <10. This indicates that shelf-life quality of canned *halang-halang* samples is still microbiologically acceptable after 15 months of storage.

IV. CONCLUSION

Among the three independent variables under study, both coconut milk, and retort time significantly affected the sensory qualities of the product, while sautéing time showed non-significant effects in all sensory qualities being evaluated. The optimum combination of the variables occurred at two different conditions. The first one requires a mid-level of coconut

milk (20%), high retort time (50 minutes) and at any sautéing time. The other one requires a combination of a mid-level of coconut milk (20%), lower retort time (30 minutes) and at any sautéing time. On the other hand, consumer preference test on the products revealed that 68% of the test consumers preferred the "hot" *halang-halang* variant than the "plain". The canned *halang-halang* samples were still shelf stable after 9 months of storage.

REFERENCES

[1]. Buzby, J.C and Farah, H.A. (2004). Chicken consumption continues longrun rise. *Diet and Health*. Retrieved on June 12, 2017. Retrieved from http://ageconsearch.umn.edu/bitstream/126587/2/ChickenConsumption.pdf

[2]. Center for Food Safety (2014). Microbiological guidelines for food (For ready-to-eat food in general and specific food items) Retrieved on August 08, 2017. Retrieved from http://www.cfs.gov.hk/english/food_leg/files/food_leg_Microbiological_Guidelines_for_Food_e.pdf

[3]. Espina, D.M. (1992). Carcass and Sensory Evaluation of the Meat Qualities of the Native Chicken and Some Locally Available Commercial Breed and their Upgrades. MS Thesis. ViSCA, Baybay, Leyte. 110 pp.

[4]. Fox, P.F., McSweeney, P.E.(2006). Advanced Dairy Chemistry *Volume 2: Lipids*. pp233

[5]. Labensky, S.R. and Hause, A.M. (1995). On Cooking. A textbook of culinary fundamentals. Prentice-Hall, Inc. Englewood Cliffs, New Jersey. 1080 pp.

[6]. Lai, P.K and Roy, J. (2004).Antimicrobial and Chemopreventive Properties of Herbs and Spices. Current Medicinal Chemistry. 2 (2) 1451-1460

[7]. Lasquites, J.M (2014, January 20) Personal Interview on how to process halang-halang

[8]. Meyer, R.H and D.C. Montgomery.(1995). Response surface Methodology: process and product optimization using designed experiments. John Wiley and Sons, Inc. New York, U.S.A. 91-99 pp.

[9]. Resureccion, A.V.A. (1998). Consumer Sensory Testing for Food Development, Aspen Publisher Incorporated. Gaithersburg, Maryland. 254 pp.

Photodynamic Effect. Experience of Application of Photosensibility Series for Monitoring Microbiological Water Pollution

© Storozok* + Nadezhda Mikhaylovna[1], Timokhina Tatyana Kharitonovna[2], Paromova Yana Igorevna[3], Voloshin Andrey Vitalevich[4]

Department of General and Bioorganic Chemistry, Department of Microbiology. Tyumen State Medical University of the Ministry of Health of Russia. ul. Odessa, 54. Tyumen, 625023. Russia.
Tel./fax: (3452) 20-74-21
* Leading direction; + Corresponding

Abstract— *The kinetics of the destruction of standard museum strains of microorganisms as a result of photodynamic action of red light and a number of non-toxic photosensitizers in the process of water conditioning has been studied experimentally. Prokaryotic cells of Escherichia coli ATCC 35218, eukaryotic cells of Candida albicans ATCC 24433 were used as the objects of the study. Eosin H, sodium fluorescein, methylene blue and riboflavin (vitamin B2) in concentrations of 10 mg/l served as photosensitizers. A photodynamic effect was established with respect to microorganism cells, leading to their death in the presence of photosensitizers and red light. It has been shown that riboflavin and fluorescein are the most effective for eukaryotes (on the example of Candida albicans ATCC 24433), which help to reduce the number of colonies of cells in 2 hours of observations by more than 3.0 and 11.0 times, respectively. It was found that the death of prokaryotic cells in the case of Escherichia coli ATCC 35218 is most effective in causing methylene blue, riboflavin (vitamin B_2). For 2 hours of observations in their presence due to photodynamic action, microflora decreases in 36.0 and 90.0 times, respectively. The photodynamic effect of eosin against the microorganisms under study was the smallest, which is explained by the peculiarities of its chemical structure, including phenolic groups, which are known to exhibit an antioxidant effect. It is shown that fluorescein and methylene blue are most promising for effective lethal action against pathogenic microflora in pool water. Riboflavin is most effective for purification of drinking water used for cooking and drinking in public, including pre-school and school meals, which will allow not only to exclude the possibility of mass poisonings, but also to provide a daily intake of vitamin B_2 with a glass of water.*

Keywords— photodynamic effect, methylene blue, riboflavin, fluorescein, red light, death of museum strains of Escherichia coli ATCC 35218, Candida albicans ATCC 24433.

I. INTRODUCTION

Water used for food purposes requires special water treatment procedures that ensure the death of microorganisms. One of the new promising ways to combat microbiological water pollution along with chlorination and ozonation is considered a photodynamic method involving the use of light and photosensitizers. The method is based on photo induced by the sensitizer the formation of active forms of oxygen, which due to the activation of free radical processes cause the death of microorganisms.

The beginning of studies of the photodynamic effect is connected with the works of O. Raab and G. von Thappeyner [1, 2], who in 1897 discovered that infusoria and other protozoans, stained with acridine derivatives, stop their growth and die under illumination. This phenomenon was called the photodynamic effect (action) (FD), which denoted the influence of light on the dynamics of cell growth, their mobility and death. It was soon shown that. For photodynamic damage of cells, in addition to the dye and light, oxygen is needed. The photodynamic effect is found in all living organisms. During the 20th century, primary mechanisms of photodynamic cell death were studied [3, 4]. It is shown that multiple lesions are induced in procaryotes as a result of photodynamic action: loss of ability to form colonies, damage to DNA, proteins, cell membranes. For the manifestation of the photodynamic effect, the presence of a photosensitizer is necessary, which increases the sensitivity of tissues and cells to light. The critical effect of the photosensitizer is the formation of active forms of oxygen in

the body, the action of which as a result of photooxidation of most biologically significant structures: amino acids (methionine, histidine, tryptophan, etc.), nucleosides, lipids, polysaccharides leads to damage and cell death.

There are two types of photodynamic processes. In the photodynamic effect of type I, the photoexcited molecules of the sensitizers of S pass into the excited singlet state of 1S * and then into the long-lived triplet state of 3T * and react with the substrate RH and the molecules of the medium, in particular, with water. Intermediate free-radical intermediates are formed, which then interact with oxygen and give a complex mixture of highly active products of a radical nature that continue reactions of free radical oxidation and damage biostructures. One of the damaging factors is singlet oxygen 1O2, which can destroy cells in the immediate vicinity of the photosensitizer molecules. Oxidizing ability of singlet oxygen is 2 orders of magnitude higher than that of normal oxygen. It can damage all the major components of cells. In nucleic acids, it attacks mainly a pair of thymine and uracil, and also causes cross-linking of DNA-DNA or DNA-protein, single-strand breaks of DNA. These effects are exacerbated by the fact that enzymes that repair DNA are particularly sensitive to singlet oxygen. However, in interphase cells, DNA is not a primary target for PD effects, since photosensitizers usually localize in the cytoplasm and do not penetrate the nucleus [3, 4]. In proteins, disulfide bonds, cysteine, histidine, tyrosine, tryptophan and phenylalanine are most easily photocontained, especially if they are located on the surface of globules and are accessible to the photosensitizer. They usually play a key role in enzymatic activity, and therefore proteins are very sensitive to photodynamic effects. Proteins lose activity as a result of photoinduced disruption of the structure of the active site, internal cross-links or intermolecular cross-links with other proteins, lipids, RNA and DNA. In type I photodynamic reactions, the radical pairs formed during electron transfer are relatively stable in an aqueous medium, where the reverse electron transport is difficult. In nonpolar lipid media, the lifetime and solubility of IO2 are higher. Consequently, type I photodynamic reactions are easier in the cytosol, and type II in the lipid phase of biomembranes. Thus, the photodynamic reactions with the participation of hydrophilic photosensitizers predominantly proceed according to the first type, and the hydrophobic photosensitizers according to the second type. Type II reactions dominate the damaging effects of most photosensitizers, including porphyrins, chlorins, phthalocyanines, and so on.

The development of oxidative stress, the disruption of the functions of cells and, as a result, their death are due to the intense generation of reactive oxygen species: superoxide radical anion (O2-), hydroxyl, hydroperoxyl radicals (OH •, HO2 •), hydrogen peroxide (H2O2), singlet oxygen (IO2). The photodynamic effect manifests itself both with UV irradiation, but especially light acts in the red wavelength range (620-780) nm. Since 1903, the study of the potential therapeutic value of the photodynamic effect began, the skin cancer was first cured with eosin staining and bright sunlight [5], since 1970 photodynamic therapy has been widely used to treat tumors [6, 7], in treatment of periodontal diseases [8-15].

Methylene green, acridine orange and proflavine, methylene blue and toluidine blue, indocyanine green, Bengal pink, eosin, curcumin, chlorine, porphyrins, phthalocyanines, chalcogen-containing benzophenoxazinium dyes, conjugates of nanoparticles with methylene blue, porphyrin or chlorine are used as photosensitizers. Cationic photosensitizers are most effective, since a positive charge enhances the interaction of the dye with the negatively charged surface of the microorganism.

In recent years, the prospects for using the photodynamic effect for purifying drinking water, controlling microbial contamination of water in aquariums and basins have been extensively studied [16-21].

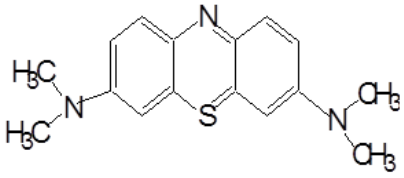
This paper presents the experience of inactivation of a number of microorganisms in water due to the photodynamic effect using red light and a number of non-toxic photosensitizers in the process of water conditioning for food purposes.

The dynamics of growth and death of standard museum strains of microorganisms of different types: prokaryotic cells of Escherichia coli ATCC 35218, and also cells of eukaryotes Candida albicans ATCC 24433 were studied.

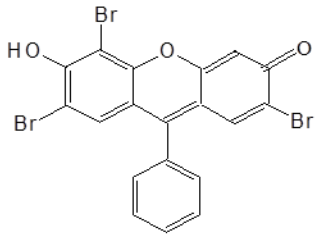
The bacterium Escherichia coli (E. coli) is found in the intestines of humans and warm-blooded animals. Most strains of E. coli are harmless, but some strains, for example, O157: H7, O121, O104: H4 and O104: H21, synthesize potentially deadly toxins that can contribute to human infection by nutritional methods with low food hygiene. The ability of virulent strains to survive for some time in the environment makes them an important indicator for investigating the presence of traces of fecal contamination in water.

Representatives of the genus Candida (primarily Candida albicans) are classified as conditionally pathogenic varieties of fungal infection. Microorganisms of the genus Candida are part of the normal microflora of the mouth, esophagus, vagina and large intestine of most healthy people. The disease is caused not only by the presence of fungi of the genus Candida, but by their multiplication in large numbers or by the entry of more pathogenic strains of the fungus. Most often, candidiasis occurs in people with a decrease in general and local immunity.

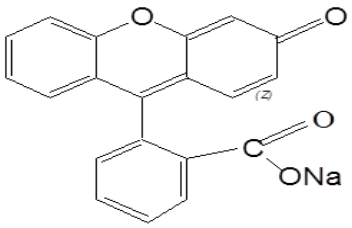
Methylene blue (I), eosin (II), sodium fluorescein (III) and riboflavin (vitamin B2) (IV) were used as sensitizers. The compound formulas are shown in Scheme 1.



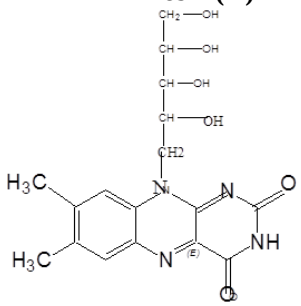
Methylene blue (I)



Eosin (II)



Fluorescein sodium (III)



Riboflavin (vitamin B_2) (IV)

SCHEME 1. FORMULAS OF PHOTOSENSITIZERS

The compounds studied are used in medicine, as a rule, as antiseptic, antimicrobial agents. Fluorescein is widely used as a diagnostic tool for the detection of lesions of the cornea of the eye, as a preparation for fluorescent angiography [22].

II. EXPERIMENTAL PART

In sterile tubes, a working concentration of microorganisms (1.5 kd / ml Candida albicans ATCC 24433 and 75.0 kts / ml Escherichia coli ATCC 35218) was prepared. One of the photosensitizers, methylene blue (I), eosin (II), fluorescein (III) and riboflavin (IV), and a culture of microorganisms were added to the flasks with sterile saline solution at comparable concentrations of 10 mg / l. The contents of the reaction vessels were mixed, which provided a high concentration of oxygen in the liquid.

The flasks were placed under a lamp emitting red light with a wavelength (620-780 nm), with a power of 250 watts. From prototypes (irradiation with light in the presence of photosensitizer) and control (red light irradiation) at 2, 5, 10, 20, 30, 40, 60 and 120 minutes, 0.1 ml samples were taken by a sterile pipette, introduced into sterile Petri plates with meat-peptone agar and thoroughly rubbed over the entire surface with a sterile spatula. The crops were incubated in a thermostat for 24 hours. CFU counts were made on the surface of the agar. In each of the control points, the reliability of the difference in the mean indicators was determined by the Student's criterion [23].

Results and its discussion When studying the effect of photosensitizers on Candida albicans ATCC 24433 and Escherichia coli ATCC 35218, it was shown that, without irradiation, the number of microorganisms of Candida albicans decreased by 10-12% (Figure 1a, curve 1) for 2 hours of observation, for Escherichia coli by 20% (Figure 1b, curve 1). When samples are exposed in a red light flux without a photosensitizer, the number of colonies decreases by 3.0 and 1.5 times, respectively, for the mentioned microorganisms (Fig. 1a, curve 2, Fig. 1b, curve 2).

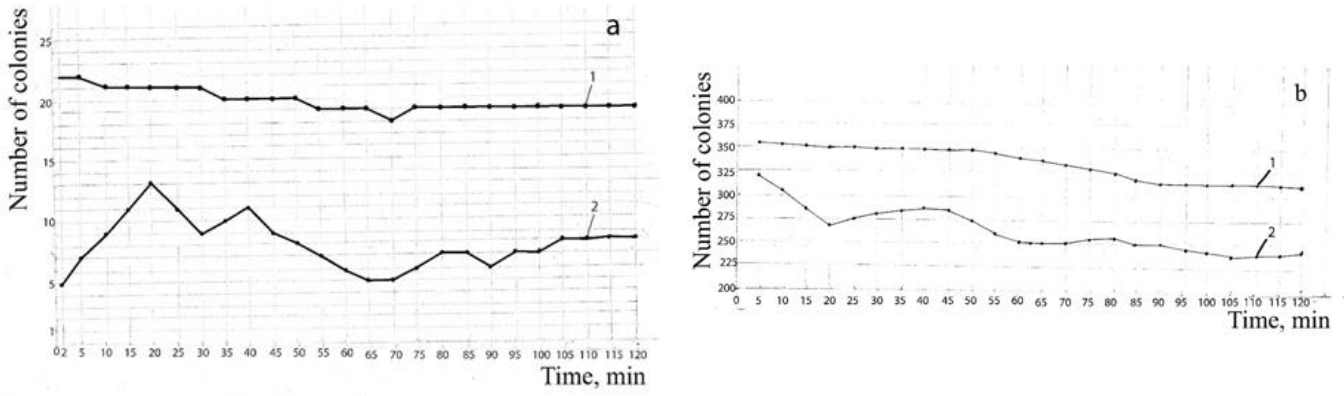


FIG. 1. A) DYNAMICS OF CHANGES IN THE NUMBER OF MICROORGANISMS CANDIDA ALBICANS ATCC 24433

Under the influence of red light. The initial concentration is 1.5 thousand / ml, T = 20 ° C, the stirring speed is 100 rpm; b) Dynamics of changes in the number of microorganisms of Escherichia coli ATCC 35218 under the action of red light. The initial concentration is 75 thousand / ml, T = 20 ° C, the stirring speed is 100 rpm.

Reducing the number of colonies of microorganisms under the influence of red color is explained by the photodynamic effect caused by the presence in the cells of endogenous photosensitizers, which include hemoglobin, porphyrins, which form part of a number of enzymes and vitamins.

In the presence of exogenous photosensitizers, there is a more significant decrease in the number of microorganisms. Comparison of a number of photosensitizers made it possible to establish differences in their action. Photo 1 shows the number of colonies of Candida albicans ATCC 24433 at 2 hours exposure in red light in the presence of fluoscecein (III), eosin (II) and methylene blue (I).

Photo 1 shows the photodynamic effect of fluorescein in comparison with culture control without irradiation and control after 2 min and 2 hours of irradiation.

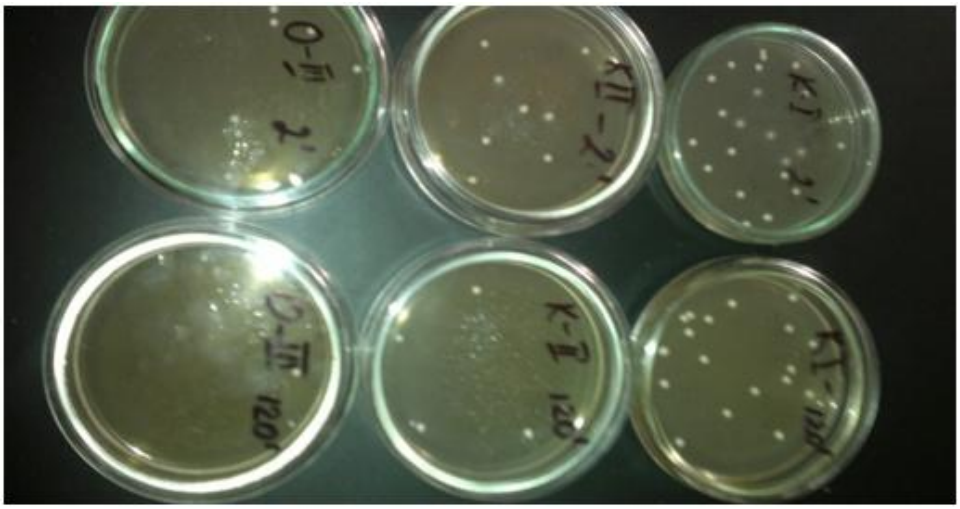


PHOTO 1. Influence of fluorescein (III) (left) upon irradiation with red light on the growth dynamics of colonies Candida albicans ATCC 24433. The concentration of the photosensitizer is 0.10 mg / l; Exposure time 2 hours. Red light (λ = 620-780 nm), lamp power 250 watts. T = 20 ° C, stirring speed 100 rpm.

A study of the growth kinetics of eukaryotic cells of Candida albicans ATCC 24433 in the presence of the photosensitizers under study showed that PD exhibited all the dyes (Fig. 2a, b).

From Fig. 2a, it is seen that eosin (II) was the least effective. Obviously, this is due to the peculiarities of the chemical structure of the compound. In the structure of eosin there are phenolic groups, which are known to act as antioxidants, capable of directly interacting with free radicals [24, 25], which leads to a decrease in the intensity of free radical processes. Reducing the number of reactive oxygen species leads to a decrease in PD. The effect of methylene blue (I) and riboflavin

(IV) is comparable, fluorescein (III) is the most effective, leading to almost complete death of microorganisms in 2 hours (Fig. 2a). The remaining dyes indicated above were inferior to fluorescein on average by 30% (Fig. 2).

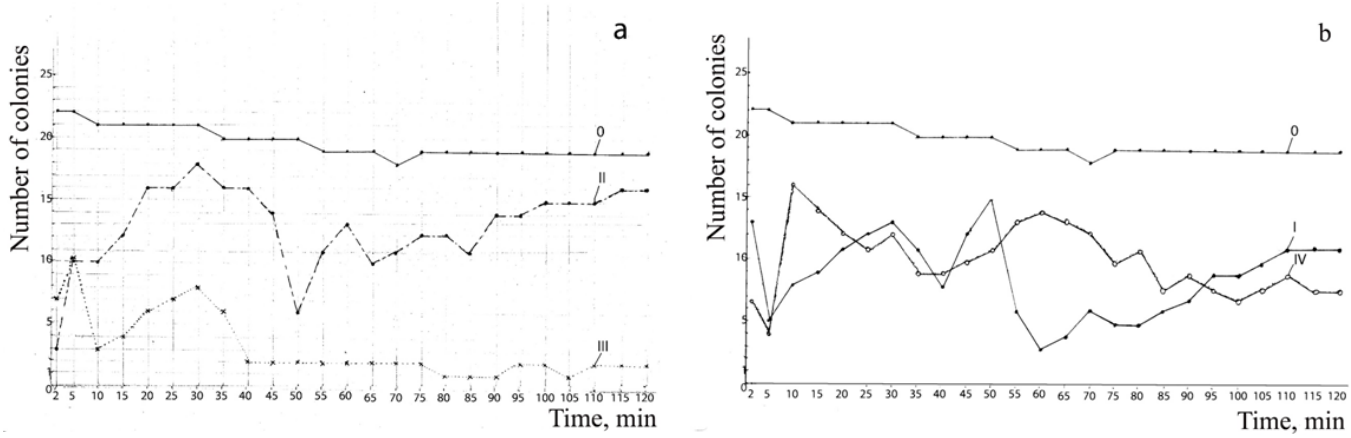


FIG. 2. a) Dependence of changes in the number of Candida albicans ATCC 24433 cells (initial concentration 1.5 kd / ml), without dye (0), in the presence of eosin (II), fluorescein (III). Irradiation with red light (λ = 620-780 nm), lamp power 250 W. The concentration of the photosensitizer is 0.10 mg / ml; T = 20 ° C, stirring speed 100 rpm. b) Dependence of changes in the number of cells of Candida albicans ATCC 24433 (initial concentration 1.5 thousand / ml), without dye (0), in the presence of methylene blue (I), riboflavin (IV). Irradiation with red light (λ = 620-780 nm), lamp power 250 watts. The concentration of the photosensitizer is 0.10 mg / l; T = 20 ° C, stirring speed 100 rpm.

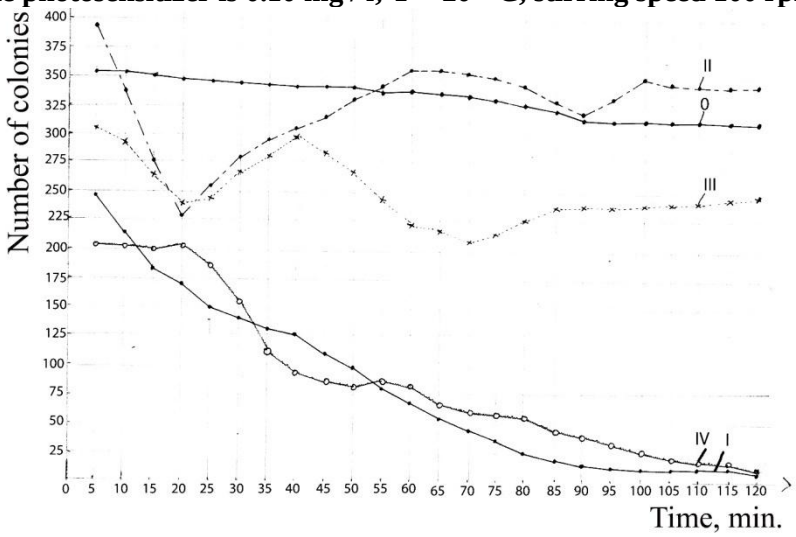


FIG. 3. Dependence of changes in the number of cells of Escherichia coli ATCC 35218,initial concentration 75 thousand / ml, without dye (0), in the presence of methyleneblue (I), eosin (II), fluorescein (III), riboflavin (IV). Concentration photosensitizer 100 mg / l; Irradiation with red light (λ = 620-780 nm), the lamp power is 250 watts. T = 20 ° C, stirring speed 100 rpm.

From the analysis of Fig. 3 it follows that eosin causes the death of Escherichia coli cells of ATCC 35218 only in the first 20 minutes of observations, after an hour and until the end of observations their amount becomes higher than in the control. The reasons for the low efficiency of eosin are explained by the inhibitory effect of the phenolic group inhibiting the development of free radical oxidation [24, 25], and, consequently, the PD photosensitizer.

A study of PD of a number of photosensitizers found that fluorescein for Escherichia coli ATCC 35218 at a concentration of 100 mg / l is significantly less effective than at a concentration of 10 mg / l for Candida albicans ATCC 24433 cells. Thus, for 2 hours of observation, the number of prokaryotic cells decreases in 1.5 times, whereas under the same conditions the level of eukaryotes decreases by 11.0 times. The disadvantage of fluorescein is its low solubility in water and the appearance of the color of the solution even at a low concentration of 10 mg / l. These properties reduce the prospects for its use for water disinfection in swimming pools and for water treatment purposes for food purposes.

With respect to Escherichia coli, ATCC 35218 methylene blue and riboflavin, introduced at a concentration of 100 mg / l, have the most effective lethal effect (Figure 3). These photosensitizers destroy practically 100% of microorganisms introduced in relatively high concentrations of 75,000 / ml for 2 hours of observation (Fig. 3). It should be noted that for natural reservoirs, lower concentrations of sensitizers are usually used, the single application is 1.0-2.5 mg / l [18], significant suppression of the number of pathogenic microorganisms is achieved with repeated application of the photosensitizer for a long time (7-28 days).

The photosensitizers under investigation are expediently used for rapid photodynamic disinfection of water. In the process of water treatment of water used for food purposes in children's sanatoriums, school and preschool institutions, riboflavin (vitamin B2) is the most promising. The compound is an important essential factor, regulates protein, carbohydrate, lipid metabolism, supports the visual function of the eye, is part of flavoproteins, which are an important link in the electron transport chain, regulate redox processes. The daily intake of vitamin B2 is difficult to obtain with food, since in most foods it is present in low amounts. When using water with riboflavin at a concentration of 10 mg / l, the daily norm of a vitamin can be obtained by a child, using 200 ml, and an adult - 300 ml. Taking more riboflavin 5-10 mg / day is used for medicinal purposes for a long time (up to six months), overdoses do not pose a danger, since vitamin B2 refers to water-soluble vitamins and is eliminated from the body during the day. Thus, for complete destruction of microorganisms in drinking water, it is advisable to use riboflavin with stirring for 2 hours and simultaneous irradiation with red light.

III. CONCLUSION

Red light in the absence of photosensitizers by 10-20% reduces the number of museum strains of microorganisms: eukaryotes of Candida albicans ATCC 24433 and prokaryotes of Escherichia coli ATCC 35218.

All studied sensitizers exhibit photodynamic action, which manifests itself after 2 min of observation. For Candida albicans ATCC 24433 riboflavin and fluorescein produce the most lethal effect and reduce the number of colonies in 2 hours of exposure in a red light flux of 3.0 and 11.0 times, respectively.

Eosin against Candida albicans ATCC 24433 and Escherichia coli ATCC 35218 exhibited a low photodynamic effect, which is explained by the peculiarities of its chemical structure.

With respect to Escherichia coli ATCC 35218 riboflavin and methylene blue, introduced at a concentration of 100 mg / L are most effective, almost 100% of microorganisms are destroyed in 2 hours of observation. Riboflavin (vitamin B2) is promising for usage in the technology of special water treatment for preschool and school meals.

REFERENCES

[1] Uber die WircingfluorescrenderStoffe auf InfusorienRaab O. Z. Biol. **1900**. Vol.39. P.524-546.

[2] The rapeutischeVersuchemitfluoreszierendenStoffenVonTappeiner, H. H. Von Tappeiner, A. JesionekMuench. Med. Wochenschr. **1903**. Vol.47. P.2042-2044.

[3] Photodynamic Action and Singential Oxygen Krasnovsky AA ml. Biophysics. **2004**. T.49. №2. P.305-321.

[4] VladimirovYu.A., PotapenkoA.Ya. Physico-chemical basis of photobiological processes M. Drofa. **2016**. 285c.

[5] ZurBehadlung der HautcarcinomemitfluorescienzendenStoffenJodlbauer A., Tappeiner H. Detsh. Arch.Med. **1905**. B.82. P.223-226.

[6] Historical essay on the development of photodynamic therapy Stranadko, EF Laser medicine. **2002**. T.6. №1. C.4-8.

[7] The main directions of photodynamic therapy in medicine Salmin R.M. Surgery news. **2008**. № 3. P.155-162.

[8] Photodynamic therapy in stomatology Konopka K, Goslinski T. J Dent Res. **2007**. Vol.86. No.11. P.1126-1132.

[9] Treatment of periodontal diseases by photodisinfection in comparison with removal of calculus and smoothing of the root surface. Andersen R., Loebel N., Hammond D., Wilson M. J. Clin Dent. **2007**. Vol.18. No.2. P.34-38.

[10] Jepsen S. Short-term clinical effects of complex antibacterial and photodynamic therapy in periodontal treatment: a randomized clinical trial. Braun A., Dehn C., Krause F. J ClinPeriodontol. **2008**. Vol.35. No.10. P.877-84.

[11] Sensitization of pathogenic bacteria in periodontal tissues when they are formed by laser radiation of low power Wilson M., Dobson J., Sarkar S. Oral Microbiol Immunol.1**993**.Vol.8. No.3. P.182-187.

[12] Antibacterial photodynamic therapy with conservative treatment of acute periodontitis: a pilot randomized controlled clinical study of Oliveira RR, Schwartz-Filho HO, Novaes AB Jr, Taba M Jr. J Periodontol. **2007**. Vol.78. No.6. P.965-973.

[13] Photodynamic inactivation of Enterococcus faecalis in dental root canals in vitro. Foschi F., Fontana C. R., Ruggiero K., Riahi R. et al. Lasers Surg. Med. **2007**. Vol.39. No.10. P.782-787.

[14] Evaluation of photodynamic therapy using a light-emitting diode lamp against Enterococcus faecalis in extracted human teeth Rios A., He J., Glickman G. N., Spears R., Schneiderman E.D., Honeyman A.L. J Endod. **2011** .Vol.37. No.6. P.856-859.

[15] Efflux pump inhibitor potentiates antimicrobial photodynamic inactivation of Enterococcus faecalis biofilm. Kishen A., Upadya M., Tegos G.P., Hamblin M.R. PhotochemPhotobiol. **2010**. Vol.86. No.6. P.1343-1349.

[16] Photodynamic disinfection of water Kuznetsova NA, Kaliya O.L. The Chemical Journal. **2013**. T.54. №2. Issue 1. P.100-109.

[17] Investigation of the toxicity of substances - sensitizers of photodynamic disinfection of the aquatic environment in a two-species test system Ipatova VI ProkhodskayaV.Yu., Dmitrieva A.G. Materials of the international conference "The current state of aquatic biological resources of marine and fresh water ecosystems in Russia: problems and solutions. Rostov on the Don. **2010**. P.86-92.

[18] Comparative analysis of toxicity of methylene blue and proflavine for saprophytic bacteria and photodynamic water disinfection Perkhvanidze VA, SimakovYu.G., Batkayeva NV General Biology. A series of natural and technical sciences. **2017**. №6. C.3-9.

[19] Physicochemical basis of sensitized phthalocyanine and acridine derivatives of photoinactivation of microorganisms in aqueous media. Makarov D.A. Abstract. Ph.D. M. **2012**. 25s.

[20] Method of photodynamic inactivation of bacteria. Zaporozhtseva ZV, Podsosonny VA, Zrodnikov VS The patent of the Russian Federation 2316366. publ. **10.02.2008.**

[21] The method of water disinfection. Kuznetsova NA, Slivka LK, Pleshkov GM, Makarov DA The patent of the Russian Federation is 2358909. publ. **20.06.2009**.

[22] Mashkovsky M.D. Medicines, in 2 tons Moscow: New Wave LLC. **2002**. 608p.

[23] Statistical analysis in medicine and biology. Tasks, terminology, logic, computer methods. Platonov AE Moscow: RAMS. **2000**. 51c.

[24] The relationship between the chemical structure and the inhibitory effect of sterically hindered phenols in the oxidation of methyloleate in a homogeneous and microheterogeneous system. Strozhok NM, Perevozkina MG, Nikiforov GA IzvestiyaAkademiiNauk. A series of chemical. **2005**. № 2. C.323-328.

[25] Interrelation of the chemical structure and antioxidant properties of N-substituted amides of salicylic acid Storozhok NM, Medyanik NP, Krysin AP, Pozdnyakov AP, Krekov SA Kinetics and catalysis. **2012**. T.53. №2. P.170-180.

Screening of Maize Genotypes against Southern Leaf Blight (Bipolaris Maydis) during summer in Rampur, Chitwan

Prakriti Sharma[1], Sallu Nepal[2], Sareeta Paudel[3], Srijana Bhandari[4], Sujata K.C[5], Sundar Man Shrestha[6]

Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal

Abstract**—** *Screening of 20 maize genotypes against Southern Leaf Blight of maize (Bipolaris maydis (Nisik) Shoemaker) was carried out in RCBD in National Maize Research Program, Rampur, Nepal during June to September, 2015. The area of research field was 315 m^2. Each genotype had two rows per plot of 7.5m^2 with two replication. Disease incidence was taken for three times at 43, 53 and 63 DAS. Disease scoring was done as percentage of leaf area infected on individual plant at 7 days interval starting from 58 days after sowing for 5 times and disease severity and mean AUDPC was calculated. Also the yield was calculated. Shade house experiment was carried out in a Completely Randomized Design with 3 replication on 20 genotypes by artificial inoculation at 3-4 leaf stage with a pure culture suspension of Bipolaris maydis (4x10^4conidia per ml) .and disease incidence and survival days of plant were recorded. Among the genotypes disease severity varied in the field. Highly significant differences were observed among the genotypes for Southern Leaf Blight severity, Area Under Disease Progress Curve and grain yield. Genotypes with mean AUDPC values from 200-250, 250-300 and above 300 were categorized as moderately resistant, moderately susceptible and susceptible. Disease severity was highest on genotype Rampur 24, 07 SADVI and lowest on BGBYPOP, RML-32/RM-17 and RAMS03F08. Highest maize yield (4.44 ton/ha) was recorded on RML-32/RML-17 and least (1.41 ton/ha) was obtained in ZM-627.In shade house, Rampur-24 followed by 07 SADVI, Rampur 27 died earlier and RML-32/RML-17 and BGBYPOP survived to the longest periods after inoculation. Disease Susceptibility pattern was similar in both field and shade house condition. The genotypes RML-32/RML-17, BGBYPOP, RAMS03F08 and TLBRS07F16 could be developed as resistant varieties to Southern Leaf Blight of maize and also as high yielders during summer under Chitwan and similar conditions.*

Keywords— AUDPC, Bipolaris maydis, disease scoring, inoculation, resistant.

I. INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops of the world grown in the irrigated and rainfed areas which ranks third after wheat and rice. Due to its high potentiality than any other cereals, it is also called as a versatile and miracle crop so it is popularly known as' Queen of Cereals'(Singh, 2002).It is the second most important crop after rice in terms of area and production and productivity (2.46 ton/ha) in Nepal. There is a wide gap between potential yield of maize varieties having 6.7 t/ha (on-station experimental yield), attainable yield of about 5.7 t/ha (on farm yield with improved practices) and national yield of 2.4 t/ha [1]. Among many,the most important factor for causing this wide gap in yield is SLB of maize caused by *B.maydis* syn. *Helminthosporium maydis (Teliomorph: Cochliobolous heterostrophus*).This disease was identified in 1965 from Rampur, Chitwan for the first time in Nepal [6].There are three physiological races of *C.heterostrophus*, they are Race O, T and C. The most prevalent race is O which attacks a broad range of genotypes. In maize, one recessive major gene for resistance has been identified namely rhm 1 which confers resistance to race O of *C. heterostrophus* [3,12].In the adult plant rhm 1 confers a level of quantitative resistance[2,11].Disease data in experimental trail and disease situation in farmer's field support the need for screening the genotypes against SLB [8].The use of fungicides is costly and environment unfriendly and it is simple, effective, safe and economical to use resistant varieties for controlling this disease. In such contest, identification of resistant genotypes/varieties would be good alternatives to manage SLB. The study was conducted with following objectives

- To determine the SLB disease incidence and disease severity of maize in field under epiphytotic condition.
- To determine the seedling incidence of SLB disease under shade house condition.
- To identify resistant and susceptible genotypes of maize against SLB disease

II. MATERIAL AND METHOD

2.1 Field Experiment

Field experiment was conducted at National Maize Research Program (NMRP), Rampur, Chitwan, Nepal during summer season (June to September, 2015) under rainfed condition and shade house and lab work were conducted atInstitue of Agriculture and Animal Science (IAAS), Rampur, Chitwan The experiment was conducted in a randomized completely block design with 2 replications. Individual plot size was 1.25 m^2 (5m x 0.25m) and the area of research field was 315m^2. There were 2 rows of 5m length/plot and 75cm apart. The susceptible check farmer's local was sown on the border of both side of field to provide uniform source of inoculum to the maize plants.Analysis of variance (ANOVA) was used to test differences among the treatments and means were separated using Duncan's multiple range test (DMRT) at the 5% level of significance [5].

2.1.1 Disease assessment

Disease incidence was taken 43, 53 and 63 DAS.

2.1.2 Disease scoring

Disease scoring was started 58 days after sowing. Southern leaf blight severity was measured as percentage of leaf area infected on individual plant visually at 7 days intervals. A total of 5 scorings were done from June to August, 2015, i.e. 58DAS, 65DAS, 72DAS, 79DAS and 86DAS. Disease scoring was done on 1 – 5(CIMMYT scale) as below.

1 = Plants with one or two to few scattered lesions on lower leaves (Resistant)

2 = Moderate number of lesions on leaves, affecting <25% of the leaf area (Moderately Resistant)

3 = Abundant lesions on lower leaves, few on other leaves affecting 26-50%

leaf area (Moderately Susceptible)

4 = Lesions abundant on lower and mid leaves, extending to upper leaves

affecting 75% leaf area (Susceptible)

5 = Lesions abundant on almost all leaves, plants prematurely dried or killed with

76-100% of the leaf area affected (Highly Susceptible)

Percent disease severity was calculated using the following formula:

$$Disease\ severity(\%)\ \frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of plants observed} \times \text{maximum rating}}$$

Disease severity was calculated/plant and mean severity was computed/plot. AUDPC value was calculated by using the following formula as given by Das *et al.*(1992).

$$AUDPC = \sum_{i=1}^{n-1} [\{(Y_i + Y_{i+1})/2\} \times (t_{(i+1)} - t_i)]$$

where, Y_i = disease severity on the i^{th} date, t_i = time on which Y_i was recorded and n = number of times observations were taken. Based on mean AUDPC value, genotypes were categorized into 3 resistance level.

Mean AUDPC value	Resistance category	Code
>300	Susceptible	S
250-300	Moderately susceptible	MS
200-250	Moderately resistant	MR

2.2 Shade house experiment

For the verification of field experiment, a greenhouse study was done. Maize leaves with typical symptoms of southern leaf blight from border plant were collected from the field and pathogen was isolated to prepare pure culture for artificial

inoculation. Maize seedlings were inoculated with suspension of *B. maydis* (4×10^4conidia/ml) on 16 October 2015,12days after sowing, with the help of a hand atomizer. Disease incidence was observed 2 days after inoculation.

2.3 Statistical analysis

ANOVA and DMRT was done by using statistical software R-STAT, correlation analysis was done using MS-EXCEL 2010 and covariance analysis was done using GEN-STAT.

III. RESULT AND DISCUSSION

Twenty maize genotypes varied considerably in incidence of SLB disease at 43, 53 and 63 DAS. Disease incidence at 43 DAS was found highest in ZM 627(76.33^{a}±6.94) which was at par with P501RCO/P502RCO, ZM401 and AC9942/AC9944and, the lowest disease incidence was seen in RAMS03S08 (12.60^{g}±4.97) which was at par with BGBYPOP, RAMPUR 33, and RML32/RML17 and the result obtained in 53 DAS and 63 DAS was found non-significant. The disease severity on 79 DAS was highest in 07 SADVI (66.00^{a}±11.31) which was at par with RAMPUR 24, 05 SADVI and ZM401 and disease severity was found minimum in BGBYPOP (37^{e}±9.89) which was at par with RAMS03F08, TLBRSO7F16, and RML32/RML17.In susceptible check F. LOCAL moderate severity (46^{bcde}±0.00) was seen. In our research lowest disease severity was shown by RML32/RML-17 which was supported by Magar(2012) with severity 7.27.

TABLE 1
SLB DISEASE SEVERITY OF 20 MAIZE GENOTYPES AT RAMPUR, CHITWAN, DURING JUNE TO AUGUST 2015

Genotypes	58 DAS	65 DAS	72DAS	79 DAS	86 DAS
RAMPUR-24	28^{ab}±2.82	43±18.38	55^{a}±4.24	66^{a}±5.65	80±11.31
07 SADVI	26^{abc}±0.00	36±14.14	50^{ab}±5.65	66^{a}±11.31	81±12.72
RAMPUR-28	31^{a}±7.07	37±4.24	53^{a}±9.89	56^{abc}±5.65	62±5.65
RAMPUR-27	27^{abc}±4.24	39±9.89	49^{ab}±1.41	55^{abcd}±1.41	61±1.41
05 SADVI	26^{abc}±0.00	36±14.14	50^{ab}±5.65	66^{a}±11.31	81±12.72
ZM-401	28^{ab}±5.65	36±16.97	41^{abcd}±18.38	55^{abcd}±7.07	69±7.07
AC9942/AC9944	25^{abc}±4.24	33±4.24	44^{abcd}±2.82	55^{abcd}±1.41	69±1.41
ZM-627	21^{bc}±1.41	35±15.55	45^{abcd}±12.72	54^{abcd}±8.48	68±11.31
P501SRCO/P502SRCO	21^{bc}±1.41	33±7.07	44^{abcd}±2.82	52^{abcd}±2.82	64±11.31
RAMPUR-32	24^{abc}±0.00	35±1.41	45^{abcd}±15.55	50^{bcde}±5.65	57±9.89
RAMPUR-36	21^{bc}±1.41	32±5.65	39^{abcd}±1.41	55^{abcd}±7.07	68±0.00
RAMPUR-34	21^{bc}±1.41	34±2.82	47^{abc}±4.24	50^{bcde}±5.65	57±7.07
RAMPUR-33	22^{bc}±0.00	33±7.07	40^{abcd}±2.82	49^{bcde}±4.24	62±5.65
RAMPUR-21	22^{bc}±2.82	31±4.24	42^{abcd}±2.82	50^{bcde}±2.82	56±8.48
F.LOCAL	22^{bc}±2.82	31±4.24	36^{abcd}±5.65	46^{bcde}±0.00	55±4.24
RAMPUR COMP	22^{bc}±0.00	29±1.41	36^{abcd}±5.65	42^{bcde}±2.82	51±4.24
TLBRS07F16	21^{bc}±1.41	24±5.65	26^{d}±2.82	41^{cde}±1.41	65±32.52
RAMS03F08	21^{bc}±1.41	26±2.82	32^{bcd}±2.82	40^{de}±14.14	50±28.28
RML-32/RML-17	20^{c}±0.00	27±7.07	29^{cd}±7.07	41^{cde}±4.24	44±2.82
BGBYPOP	21^{bc}±1.41	24±2.82	26^{d}±5.65	37^{e}±9.89	44±5.65
LSD	6.03*	NS	16.11*	13.56**	NS
CV (%)	12.25	21.61	18.70	12.74	16.99
Grand mean	23.50	32.60	41.15	50.85	61.8

DAS: Days after sowing, CV: Coefficient of variation, LSD: Least significant difference: Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance, Sd (±) represents standard deviation, **:Highly significant, *: Significant, NS: Non significant

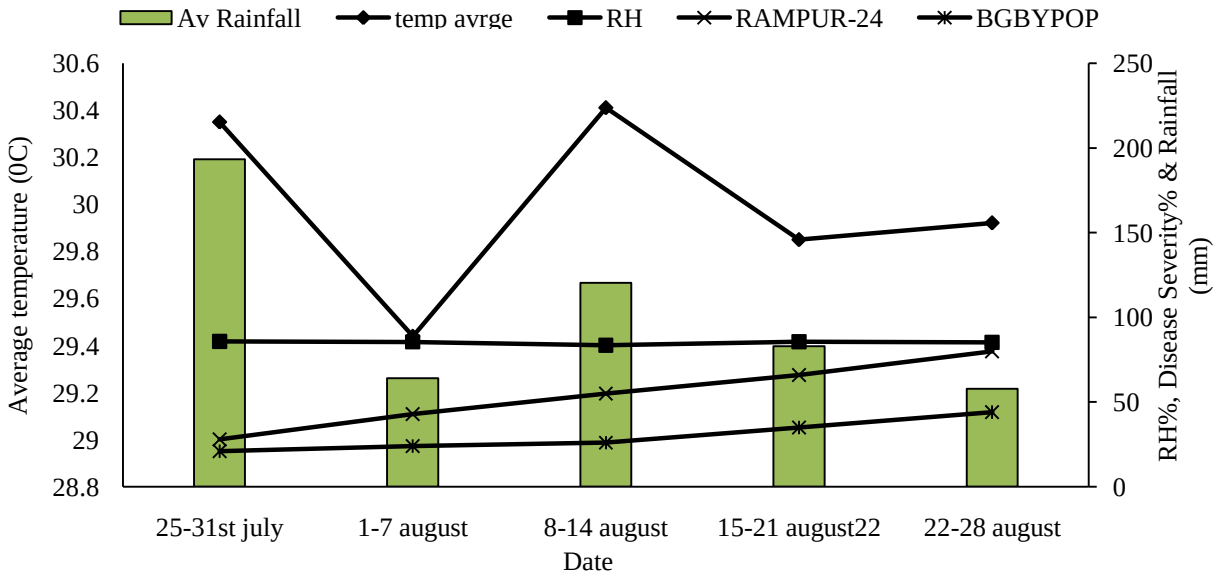


FIGURE 1. METEOROLOGICAL FACTORS AND DISEASE SEVERITY OF RAMPUR-24 AND BGYPOP

TABLE 2
AUDPC VALUES OF SLB ON 20 MAIZE GENOTYPES IN FIELD AT RAMPUR, CHITWAN, JUNE TO AUGUST 2015

Genotypes	AUDPC1 (58 DAS)	AUDPC2 (65DAS)	AUDPC3 (72DAS)	AUDPC4 (79DAS)	Total AUDPC
RAMPUR-24	248.5±54.44	343.0±79.19	423.5^{a}±34.64	511.0^{ab}±59.39	1526.0^{a}±227.68
07 SADVI	217.0±49.49	301.0±69.29	406.0^{ab}±19.79	514.5^{a}±84.14	1438.5^{ab}±14.84
RAMPUR-28	238.0±9.89	315.0±19.79	381.5^{abc}±54.44	413.0^{abcde}±39.59	1347.5^{abc}±123.74
RAMPUR-27	231.0±19.79	308.0±39.59	364.0^{abcd}±0.00	406.0^{abcdef}±9.89	1309.0^{abcd}±79.19
05 SADVI	217.0±39.59	273.0±49.49	353.5^{abcde}±4.94	455.0^{abc}±59.39	1291.5^{abcd}±34.64
ZM-401	224.0±79.19	269.5±123.74	$336.0^{abcdefg}$±89.09	434.0^{abcd}±0.00	1263.5^{abcde}±292.03
AC9942/AC9944	203.0±0.00	269.5±4.94	346.5^{abcdef}±4.94	434.0^{abcd}±0.00	1253.0^{abcde}±0.00
ZM-627	196.0±49.49	280.0±98.99	346.5^{abcdef}±74.24	427.0^{abcd}±9.89	1249.5^{abcde}±212.83
P501SRCO/P502SRCO	189.0±19.79	269.5±34.64	$336.0^{abcdefg}$±19.79	406.0^{abcdef}±29.69	1200^{abcdef}±44.54
RAMPUR-32	206.5±4.94	280.0±59.39	332.5^{abcdef}±74.24	374.5^{cdef}±54.44	1193.5^{abcdef}±193.04
RAMPUR-36	185.5±14.84	248.5±14.84	$329.0^{abcdefg}$±29.69	430.5^{abcd}±24.74	1193.5^{abcdef}±24.74
RAMPUR-34	192.5±14.84	283.5±4.94	339.5^{abcdef}±34.64	374.5^{cdef}±44.54	1190.0abcdef±69.29
RAMPUR-33	192.5± 24.74	255.5±34.64	$311.5^{bcdefgh}$±24.74	388.5^{bcdef}±34.64	1148.0^{bcdefg}±118.79
RAMPUR-21	185.5±24.74	255.5±24.74	$322.0^{abcdefgh}$±19.79	371.0^{cdef}±39.59	1134.0^{bcdefg}±108.89
F.LOCAL	185.5±4.94	234.5±34.64	287.0^{cdefgh}±19.79	353.5^{cdef}±14.84	1060.5^{cdefg}±44.54
RAMPUR COMP	178.5±4.94	227.5±24.74	273.0^{defgh}±29.69	325.5^{def}±24.74	1004.5^{defg}±84.14
TLBRS07F16	157.5±24.74	175.0±29.69	234.5^{gh}±14.84	371.0^{cdef}±108.89	938.0^{efg}±39.59
RAMS03F08	164.5±14.84	203.0±19.19	252.0^{efgh}±59.39	315.0^{def}±148.49	934.5^{efg}±242.53
RML-32/RML-17	164.5±24.74	196.0±49.49	245.0^{fgh}±9.89	297.5^{ef}±24.74	903.0^{fg}±59.39
BGBYPOP	157.5±4.94	175.0±29.69	220.5^{h}±54.44	283.5^{f}±54.44	836.5^{g}±143.54
LSD	NS	NS	90.00259**	107.2892*	290.578**
CV (%)	14.71	18.66	13.35	13.00	11.85
Grand Mean	196.35	258.125	322	394.275	1170.75

DAS: Days after sowing, CV: Coefficient of variation, LSD: Least significant difference: Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance, Sd (±) represents standard deviation, **:Highly significant, *: Significant, NS: Non significant

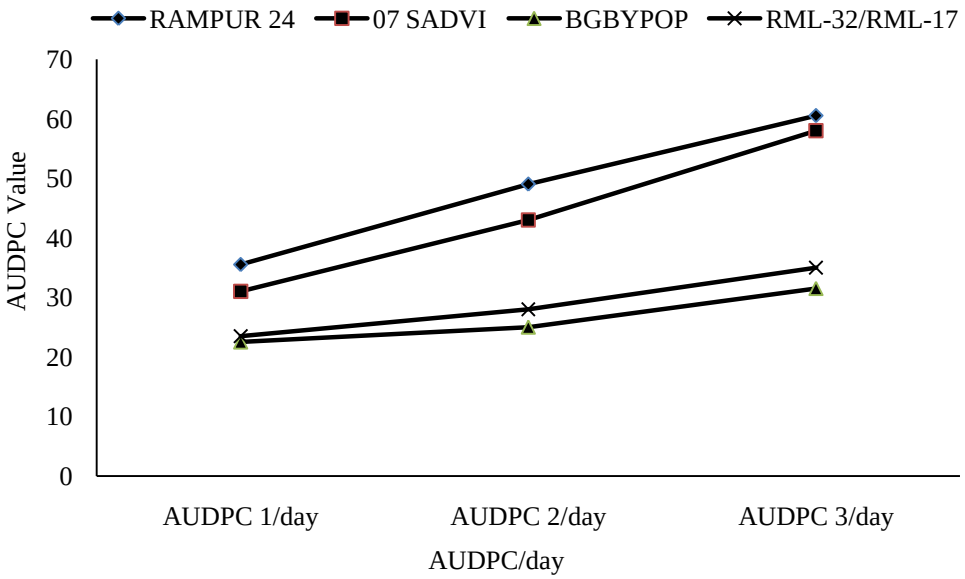


FIGURE 2. AUDPC VALUE OF DIFFERENT GENOTYPES

3.1 Area under disease progress curve (AUDPC)

The AUDPC1, AUDPC2, AUDPC3, AUDPC4 and total AUDPC were computed for all the genotypes expressing the disease severity (Table 3). The AUDPC1 58 days after sowing were found non-significant. Similarly, AUDPC2 also found non-significant. The AUDPC3 72 days after sowing was found to be highly significant. The genotype BGBYPOP had the lowest AUDPC which was at par with TLBRS07F16, RML-32/RML-17 and RAMS03F08 and highest AUDPC3 was found in RAMPUR-24 which was at par with 07SADVI, RAMPUR-28, RAMPUR-27.The AUDPC4 was found lowest in the genotype BGBYPOP which was at par with RML-32/RML-17, RAMS03F08 and the highest AUDPC was found in the genotype 07 SADVI which was at par with RAMPUR-24, 05 SADVI.

TABLE 3

RESISTANCE CATEGORY OF 20 MAIZE GENOTYPES ON THE BASIS OF MEAN AUDPC AND AUDPC PER DAY VALUES IN FIELD AT RAMPUR, CHITWAN, DURING JUNE TO AUGUST, 2015

Genotypes	Mean AUDPC	AUDPC per Day	Resistance category	Number of genotypes
RAMPUR-24	381.5^{a}±56.92	54.50^{a}±8.13	S	9
07 SADVI	359.62^{ab}±3.71	51.37^{ab}±0.53	S	
RAMPUR-28	336.87^{abc}±30.93	48.12^{abc}±4.41	S	
RAMPUR-27	327.25^{abcd}±19.79	46.75^{abcd}±2.82	S	
05 SADVI	322.87^{abcd}±8.66	46.12^{abcd}±1.23	S	
ZM-401	315.87^{abcde}±73.00	45.12^{abcde}±10.42	S	
AC9942/AC9944	313.25^{abcde}±0.00	44.75^{abcde}±0.00	S	
ZM-627	312.37^{abcde}±53.20	44.62^{abcde}±7.60	S	
P501SRCO/P502SRCO	300.12^{abcdef}±11.13	42.87^{abcdef}±1.59	S	
RAMPUR-32	298.37^{abcdef}±48.26	42.62^{abcdef}±6.89	MS	7
RAMPUR-36	298.37^{abcdef}±6.18	42.62^{abcdef}±0.88	MS	
RAMPUR-34	297.50^{abcdef}±17.32	42.50^{abcdef}±2.47	MS	
RAMPUR-33	287.00^{bcdefg}±29.69	41.00^{bcdefg}±4.24	MS	
RAMPUR-21	283.50^{bcdefg}±27.22	40.50^{bcdefg}±3.88	MS	
F.LOCAL	265.12^{cdefg}±11.13	37.87^{cdefg}±1.59	MS	
RAMPUR COMP	251.12^{defg}±21.03	35.87^{defg}±3.00	MS	
TLBRS07F16	234.50^{efg}±9.89	33.50^{efg}±1.41	MR	4
RAMS03F08	233.62^{efg}±60.63	33.37^{efg}±8.66	MR	
RML-32/RML-17	225.75^{fg}±14.84	32.25^{fg}±2.12	MR	
BGBYPOP	209.12^{g}±35.88	29.87^{g}±5.12	MR	
LSD	72.63**	10.375**		
CV (%)	11.85	11.85		
Grand Mean	292.68	41.8125		

The genotype RAMPUR-24 had the highest total AUDPC which was at par with 07SADVI, RAMPUR-28 and the lowest total AUDPC was obtained in the genotype BGBYPOP which was at par with RML-32/RML-17, RAMS03F08.

3.2 Categorization of genotypes based on mean AUDPC

The mean AUDPC value ranged from (209.12±35.88) to (381.5±56.92) (Table 4) which differ highly significantly from each other. The genotype BGBYPOP had the lowest AUDPC which was at par with RML-32/RML-17 and RAMPUR 24 had the highest mean AUDPC which was at par with 07SADVI. Similar observations were found for AUDPC per day values. The genotypes RML-32/RML-17 (32.25±2.12), RAMS03F08 (33.37±8.66) did not differ significantly from BGBYPOP (29.87±5.12).

TABLE 4
MEAN YIELD DATA OF 20 MAIZE GENOTYPES AT RAMPUR, CHITWAN, 2015

Genotypes	Yield (t/ha)	Yield after covariance analysis
RAMPUR-24	1.59^{d}±0.51	2.109^{cde}±0.51
07 SADVI	2.23^{bcd}±0.14	2.058^{cde}±0.14
RAMPUR-28	3.80^{ab}±1.97	3.682^{ab}±1.97
RAMPUR-27	3.55^{ab}±0.49	3.089^{bcd}±0.49
05 SADVI	2.69^{abcd}±0.07	2.690^{bcde}±0.07
ZM-401	2.25^{bcd}±0.86	2.481^{bcde}±0.86
AC9942/AC9944	2.61^{abcd}±0.79	2.610^{bcde}±0.79
ZM-627	1.41^{d}±0.13	1.987^{cde}±0.13
P501SRCO/P502SRCO	2.06^{bcd}±0.09	1.660^{de}±0.09
RAMPUR-32	3.51^{abc}±0.06	2.531^{bcde}±0.06
RAMPUR-36	2.88^{abcd}±1.42	3.172^{bcd}±1.42
RAMPUR-34	1.66^{cd}±0.28	1.491^{e}±0.28
RAMPUR-33	2.61^{abcd}±0.03	2.553^{bcde}±0.03
RAMPUR-21	2.28^{bcd}±0.16	2.047^{cde}±0.16
F.LOCAL	2.70^{abcd}±0.71	3.107^{bcd}±0.71
RAMPUR COMP	2.54^{bcd}±0.79	3.351^{abc}±0.79
TLBRS07F16	2.29^{abcd}±0.68	3.194^{bcd}±0.68
RAMS03F08	3.91^{ab}±0.51	3.219^{abc}±0.51
RML-32/RML-17	4.44^{a}±1.06	4.674^{a}±1.06
BGBYPOP	3.52^{abc}±0.39	3.402^{abc}±0.39
LSD	1.59*	1.3226*
CV (%)	27.66	21.9
Grand Mean	2.755	2.755

DAS: Days after sowing CV: Coefficient of variation, LSD: Least significant of difference, Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance, Sd (±) represents standard deviation.

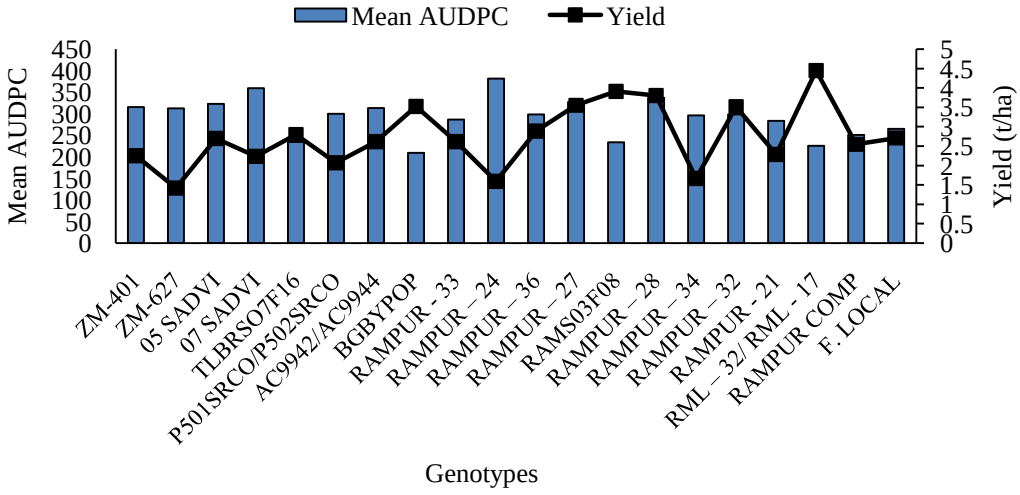


FIGURE 3 RELATION BETWEEN MEAN AUDPC AND YIELD OF GENOTYPES

Based on mean AUDPC, the genotypes were categorized as moderately resistant, moderately susceptible and susceptible against the pathogen.RAMPUR-24 and 07-SADVI was susceptible while BGBYPOP, RML-32/RML-17, RAMS03F08 and TLBRS07F16 were moderately resistant. Among the rest of the genotypes, many of them were moderately susceptible and susceptible.

3.3 Yield

Maximum grain yield (4.44 t/ha) was recorded in RML-32/RML-17 which was supported by Magar (2012).The yield of RML32/RML17 was at par with RAMS03FO8,RAMPUR-28 and BGBYPOP. The lowest grain yield was obtained in ZM-627 which was at par with the genotypes RAMPUR-24 and RAMPUR-34.

TABLE 5
DISEASE INCIDENCE ON MAIZE GENOTYPES IN SHADE HOUSE CONDITION AT RAMPUR, CHITWAN, OCTOBER, 2015

Genotypes	DI 14DAS	DI 15DAS
RAMPUR-24	41.66^{ab}±2.88	88.33^{a}±20.20
07 SADVI	49.33^{a}±5.13	93.33^{a}±11.54
RAMPUR-28	26.00^{efg}±1.73	42.33^{ef}±2.51
RAMPUR-27	30.00^{defg}±10	100.0^{a}±0.00
05 SADVI	20.66^{gh}±1.15	41.0^{ef}±7.93
ZM-401	37.00^{bcd}±2.64	91.66^{a}±14.43
AC9942/AC9944	31.66^{cde}±10.40	100^{a}±0.00
ZM-627	41.66^{ab}±10.40	100^{a}±0.00
P501SRCO/P502SRCO	38.33^{bcd}±10.40	100^{a}±0.00
RAMPUR-32	40.00^{bc}±5.00	86.66^{a}±23.09
RAMPUR-36	30.00^{defg}±0.00	63.33^{bc}±2.88
RAMPUR-34	37.33^{bcd}±4.04	68.33^{b}±2.88
RAMPUR-33	22.00^{fgh}±3.46	41.66^{ef}±6.50
RAMPUR-21	33.66^{bcde}±3.21	61.00^{bcd}±13.89
F.LOCAL	26.00^{efg}±1.73	54.66^{bcde}±0.57
RAMPUR COMP	30.66^{def}±1.15	45.00^{def}±0.00
TLBRS07F16	26.00^{efg}±1.73	57.66^{bcde}±2.51
RAMS03F08	25.33^{efg}±0.57	41.66^{ef}±5.77
RML-32/RML-17	20.66^{gh}±1.15	48.33^{cdef}±2.88
BGBYPOP	16.00^{h}±1.73	32.33^{f}±2.51
LSD	8.11***	15.38***
CV (%)	15.74	13.71
Grand Mean	31.2	67.866

DAS: Days after Sowing, CV: Coefficient of variation, LSD: Least significant of difference, Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance, Sd (±) represents standard deviation.

TABLE 6
CORRELATION BETWEEN FINAL DISEASE INCIDENCE, FINAL DISEASE SEVERITY, MEAN AUDPC AND YIELD (T/HA) AT RAMPUR, CHITWAN

Correlations				
	Final disease incidence	Final disease Severity	Mean AUDPC	Yield (ton/ha)
Final disease Incidence	1	.263	.462**	-.291
Final disease Severity		1	.664**	-.380*
Mean AUDPC			1	-.309
Yield (ton/ha)				1
**. Correlation is significant at the 0.01 level (2-tailed).				
*. Correlation is significant at the 0.05 level (2-tailed				

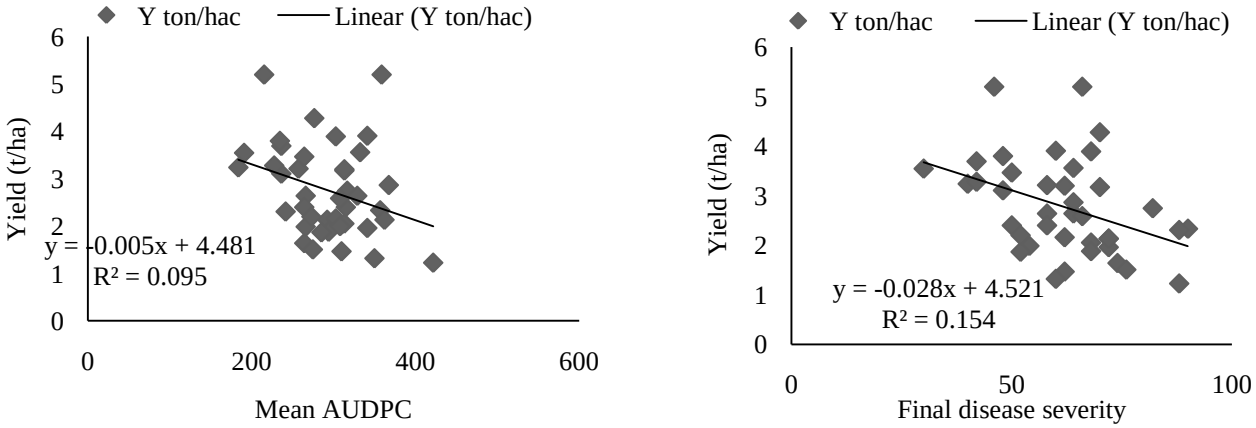


FIGURE 4. ESTIMATED LINEAR RELATIONSHIP BETWEEN YIELD AND MEAN AUDPC AND YIELD AND FINAL DISEASE SEVERITY OF 20 MAIZE GENOTYPES AT RAMPUR, CHITWAN

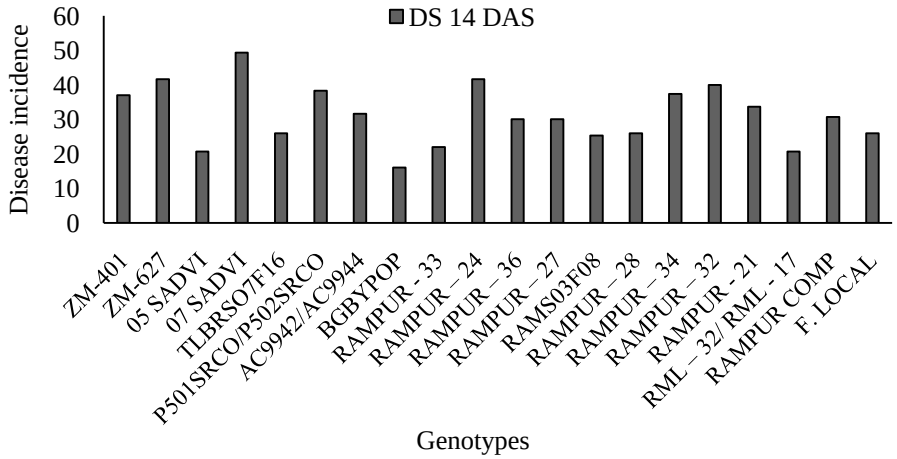


FIGURE 5 DISEASE INCIDENCE OF DIFFERENT GENOTYPES UNDER GREEN HOUSE CONDITION ON 14 DAS

IV. CONCLUSION

Among 20 maize genotypes, BGBYPOP, RML-32/RML-17 and RAMS03F08 were resistant to southern leaf blight of maize with lower disease severity and higher yield. These genotypes could be used as resistant varieties and can be used as sources of resistance for breeding. The genotypes like Rampur-24, 07 SADVI were highly susceptible to southern leaf blight of maize with maximum disease severity and mean AUDPC value. These genotypes can be used as susceptible check.Rampur-28, Rampur-27 and Rampur-32 had higher disease incidence and severity but grain yield was statistically similar with high yielders and the resistance genotypes BGBYPOP, RAMS03F08.Hence they can be used as tolerant genotypes against SLB.

ACKNOWLEDGEMENTS

We would like to acknowledge National Maize Research Program (NMRP), Rampur for proving the genotypes. Suggestions and guidance from the members of the advisory committee was highly praised. Finally we would like to thank IAAS, Pathology Laboratory and all the helping hands for making our research successful.

REFERENCES

[1]. (2014). *Annual Report 2070/71(2013/14).* National Maize Research Program,Rampur,Chitwan,Nepal.

[2]. Balint-Kurti, P. J., Zwonitzer, J. C., Wisser, R. J., Carson, M. L., Oropeza-Rosas, M. A., Holland, J. B., & Szalma, S. J. (2007). Precise Mapping of Quantitative Trait Loci for Resistance to Southern Leaf Blight, Caused by Cochliobolus heterostrophus Race O, and Flowering Time Using Advanced Intercross Maize Lines. *GENETICS, 176*(1), 645-657.

[3]. Chang, R. Y., & Peterson, P. A. (1995, March 1). Genetic Control of Resistance to Bipolaris maydis:One Gene or Two Genes? *Journal of Heredity, 86*(2), 94-97.

[4]. Das, M. K., Rajaram, S., Mundt, C. C., & kronstad, W. E. (1992). Das, M. K., Rajaram, S., Mundt, C. C., & K Inheritance of slow-rusting resistance to leaf rust in wheat. *Crop Scirence, 32*, 1452-1456.

[5]. Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agriculture research.* New York,USA: A Wile Interscience Publication.

[6]. Khadka, B. B., & Shah, S. M. (1967). Preliminary list of plant disease records in Nepal. *Nepla Journal of Agriculture, 2*, 47-76.

[7]. Magar, P. B., Khatri-Chhetri, G. B., Shrestha, S. M., & Rijal, T. R. (2015, January). Screening of Maize Genotypes against Southern Leaf Blight (Bipolaris maydis) during Summer in Rampur, Chitwan, Nepal. *Journal of Institute of Agriculture and Animal Science*, 115-122. Retrieved from ResearchGate.

[8]. Paudel, D. C., & Basnet, R. (2003). Screening of maize inbreds against southern leaf blight . *Proceedings of the 24th National Summer Crops Research Workshop,2004* (pp. 235-237). NMRP,Rampur,Chitwan.

[9]. Singh, C. (2002). *Modern techniques of raising th field crops.* New Delhi: Oxford and IBH Publishing Co.Pvt.Ltd.

[10]. Slopeck, S. W. (n.d.). An improved method of estimating percent leaf area diseased using a 1 to 5 disease scale. *Canadian journal of Plant Pathology*, 381-387.

[11]. Thompson, D. L., & Berguist, R. R. (1984). Inheritance of mature plant resistance to Helminthosporium maydis race 0 inn maize. *Crop Science, 24*, 807-811.

[1] Zaitilin, D. S., DeMars, & Y, M. (1993). Linkage of rhm,a resistance gene for resistance to Southern leaf blight,to RFLP marker loci in the maize(Zea mays) seedlings. *Genome, 36*(3), 555-564.

Evaluation of Total Petroleum Hydrocarbon (TPH) in Sediments and Aquatic macrophytes in the River Nun, Amasoma Axises, Niger Delta, Nigeria.

Alagoa, K.J[1*], Godwin, J[2], Daworiye, P.S[3], Ipiteikumoh, B[4]

[1]Department of Biological Sciences, Niger Delta University, Amasoma, Bayelsa State.
[2]Department of Chemical Sciences, Niger Delta University, Amasoma, Bayelsa State.
[3]Department of Biological Sciences, Isaac Jasper Boro College of Education, Sagbama, Bayelsa State
[4]Department of Mathematics, Isaac Jasper Boro College of Education, Sagbama, Bayelsa State.

***Abstract*—** The Total Petroleum Hydrocarbon (TPH) in the bottom sediments and aquatic macrophytes of the River Nun at AmasomaAxises was investigated in December, 2017. This was done to establish the existing levels of TPH in the River in order to ascertain the degree of its threat to the environment. Benthic samples were collected close to the shore with the aid of a hand trowel and put in aluminum foils, while macrophyte samples were collected by hand pulling and transported in plastic bags for laboratory analysis. Data were subjected to statistical analysis using the Microsoft Excell® tool pack. Regression analysis was employed in order to determine the correlation between TPH in bottom sediments with that in plant tissues (leaf, stem and root). Analysis of variance was employed at the 95% confidence level to determine the degree of significance in interaction of TPH between sediments and macrophyte tissues and between macrophyte tissues (leaf, stem, and root). Duncan multiple range test was use to compare means. The bioaccumulation factor (BAF) was calculated for TPH in order to estimate the absorption rate of TPH between sediments and plant tissues (leaf, stem and root). Results indicate that TPH were recorded in sediments and plant tissues. TPH concentrations were greater in plant tissues than in sediments. Concentration of TPH showed concentrations indicating that root> stem> leaf in most stations. The relationships between the TPH attributes indicated strong association between Leaves and stem (r^2 = 0.92). Strong negative association was also observed between sediment and stem (r^2 = -0.83) while sediment and leaves (r^2 = -0.64) had moderate negative relationship**.** It can be concluded that the River Nun at AmasomaAxises is mildly polluted due to land based activities and therefore there is a need to enact water use regulatory ordinances to protect its ecology. Sediment organisms and plants are vital links to man in the food chain. This may portend danger in the future.

***Keywords*— *Total Petroleum Hydrocarbon, Sediments, Macrophytes, Nun River. Amasoma*.**

I. INTRODUCTION

Total petroleum Hydrocarbon (TPH) is a term used for any mixture of hydrocarbons found in crude oil. They are several hundred of these compounds, but not all occur in any one sample. There are so many different chemicals in crude oil and other chemical products for instance that it is not practical to measure each one separately. Therefore the measurement of TPH becomes most apt, as it gives a definitive picture of the whole.

The contamination of aquatic ecosystems with TPH is a disturbing reality. The majority of TPH entering aquatic environments remain close to sites of deposition, suggesting that lakes, rivers, estuaries and coastal environments near centers of human population are the primary repositories of the aquatic TPH [1]. Discharges from urban catchments may carry with them sediments, nutrients, heavy metals, pesticides, oils and hydrocarbons and solid pollutants such as litter [2]. Also, Industrial discharges also carry significant TPH and thermal load to receiving environments [3].

TPH sources of pollution of the aquatic environment are so diverse that no single source can be held culpable. Sadly, the accumulation of TPH in an aquatic environment has direct consequences to man and to the ecosystem due to the intrigues of food chain [4]. This is as a result their easy affinity for bottom sediments and plant tissues. This may imply serious health implications from bioaccumulation and bio-magnifications in living organisms.

Therefore the fate of TPH introduced by human activities into aquatic ecosystems have become the subject of wide spread concern, since beyond the tolerable limits they become toxic [5, 6].The measurement of TPH in the aquatic ecosystem is a universally accepted practice for determining the pollution status and integrity of water bodies.

The River Nun at the Amasoma axis is a fresh water body and an appendage that lies along the 160km flow course of the River from its flow origins of the River Niger. It is the main live-wire of the Amasoma people as it provides their source for

fish and a transportation route for trade and commerce for goods coming and leaving this ancient city settlement. Like every threatened water body that has lots of heavy human activities and marine transport, the River is prone to TPH pollution.

Therefore there is an acute need to investigate the TPH levels in bottom sediments and aquatic macrophytes of the River. Bottom sediment pollution is considered by many regulatory agencies to be one of the largest risks to the aquatic environment, since many aquatic organisms spend the major part of their life cycle living on or in sediments [7].

This study will reveal the existing levels of TPH in the River in order to ascertain the degree of its threat to human health and the environment

II. MATERIALS AND METHODS

2.1 Study Area

The study area is located at the Amasoma Axis of River Nun. The Coordinates and description of the study stations are captured in Table 1 below

TABLE 1
DESCRIPTION OF THE STUDY AREA.

Study site	Stations	Latitude	Longitude	Description of station
River Nun at Amasoma Axis	A	$4^0$30'12''	$6^0$01'41''	Jetty area and passenger loading terminal
	B	$4^0$32'15''	$6^0$02'43''	Fish landing area for fishers the river
	C	$4^0$50'14	$6^0$05'16''	Refuse dump area of the river

2.2 Sample collection

2.2.1 Sediment Samples

Sediment samples were collected using a hand trowel to scoop sediments near the shore into polyethylene bags and bottles for PAH analysis. In each station, triplicate samples were collected and stored in an ice chest before transferring to the laboratory.

2.2.2 Aquatic Macrophyte Samples

Macrophyte samples were collected from each station by hand-pulling randomly of the free floating and fixed macrophytes, and samples were properly tagged, stored in plastic bags and then transported to the laboratory for analysis.

2.3 Sample Analysis

2.3.1 Sediment Analysis

2gm of sediment samples were weighed into a clean extraction container. 10ml of extracted solvent (pentane) was added into the samples and mixed thoroughly and allowed to settle.

The mixtures were carefully filtered into clean solvent-rinsed extraction bottles, using filter papers fitted into Buchner funnels.

The extract were concentrated to 2ml and then transferred for cleanup/separation.

2.3.1.1 Cleanup/ Separation

1cm of moderately packed glass wool was placed at the bottom of 10mm/L X250mm long chromatographic column. Slurry of 2g activated silica in 10ml methylene chloride was prepared and placed into the chromatographic column. To the top of the column was added 0.5cm of sodium sulphate. The column was rinsed with additional 10ml of methylene chloride. The column was pre-eluted with 20ml of pentane. This was allowed to flow through the column at a rate of about 2 minutes until the liquid in the column was just above the sulphate layer. Immediately, 1ml of the extracted sample was transferred into the column. The extraction bottle was rinsed with 1ml of pentane and added to the column as well.

The stop-clock of the column was opened and the eluent was collected with a 10ml graduated cylinder. Just prior to exposure of the sodium sulphate layer to air, pentane was added to the column in 1-2ml increments. Accurately measured volume of 8-10ml of the eluent was collected and labeled aliphatic (TPH).

2.3.1.2 Gas Chromatography Analysis

The concentrated aliphatic fractions were transferred into labeled glass vials with rubber crimp caps for GC analysis.1ml of the concentrated sample was injected by means of hypodermic syringe through a rubber septum into the column. Separation occurs as the vapour constituent partition between the gas and liquid phases. The sample was automatically detected as it emerges from the column (at a constant flow rate) by the FID detector whose response is dependent upon the composition of the vapour.

2.3.2 Plant TPH Analysis

Oven-dried plant material of 2gm was put in a flask and 10ml of pentane/ dichloromethane added to it as solvent. The mixture was filtered and the resulting filtrate made up to mark by the addition of distilled water. 1µl of the solution was introduced into the GC equipment using a syringe. Determination of TPH was then made.

2.4 Data Analysis

The bioaccumulation factor (BAF) was calculated for TPH in order to estimate the absorption rate of TPH between sediments and plant tissues (leaf, stem and root) using the equation below:

$$Bioaccumulation\ factor\ (BAF) = \frac{Conc\ of\ parameter\ in\ organism}{Conc\ of\ parameter\ environment}$$

Or

$$\frac{Conc\ of\ parameter\ in\ Macrophyte\ (Sample)}{Conc\ of\ parameter\ in\ Sediment}$$

Regression analysis was employed using the Microsoft Excell® tool pack in order to determine the correlation between TPH in bottom sediments with that in plant tissues (leaf, stem and root). Analysis of variance was employed at the 95% confidence level to determine the degree of significance in interaction of TPH between sediments and macrophyte tissues and between macrophyte tissues (leaf, stem, and root).

PLATE1: PICTORIAL PRESENTATION OF RIVER NUN AT AMASOMA AXIS

III. RESULT

The results of the study are captured in Tables 2 – 4.

TABLE 2
TPH (ppm [mg/kg]) IN MACROPHYTES AND SEDIMENTS IN NUN RIVER AMASOMA

Parameters	Stations ST1	ST2	ST3
Leaves	0.14^{ad}	0.14^{ab*}	0.11^{ba}
Stem	0.18^{ac}	0.18^{ac}	0.14^{bb}
Root	0.21^{ab}	0.23^{bd}	0.20^{ac}
Sediment	0.21^{ab}	0.27^{be}	0.36^{cd}

Means with the same letter superscript along the same column are not significantly different.
Means with the same letter superscript along the same row are not significantly different

TABLE 3
CORRELATION CO-EFFICIENT FOR TPH IN SEDIMENT AND MACROPHYTE (LEAVES, STEM, AND ROOT).

	Sediment	Leaves	Stem	Root
Sediment	1			
Leaves	-0.64	1		
Stem	-0.83	0.92	1	
Root	-0.16	-0.16	-0.09	1

TABLE 4
BIOACCUMULATION FACTOR FOR TPH

Parameters	Stations ST1ST2ST3		
Leaf	0.67	0.52	0.31
Stem	0.86	0.67	0.39
Root	1.0	0.85	0.56

IV. DISCUSSION AND CONCLUSION

Despite the presence of potential anthropogenic sources of TPH in the river shores at the study stations, this study found only insignificantly low concentrations of TPH in sediment and plant tissues. This is in disagreement with the findings of previous works done on similar water bodies with human inputs[8]. However, the presence of TPH may be due to their easy affinity for bottom sediments and plant tissues. Also the presence of TPH in plant tissues was observed to be correlated to the amounts of these elements in bottom sediments because plants ingest or absorb them from sediments.[9] noted that the extent of bioaccumulation in biota is dependent on the chemical effect of the metal or pollutant, its tendency to bind to particular materials and or the lipid content and composition of the biological tissues. In a similar study [10] also observed differential rate of adsorption of heavy metals in leaf, stem, and root of bitter leaf and okra plants in the Niger Delta exposed to metal polluted soils.

The study reveal a spatial increasing trend in TPH in both sediments and plant tissues from station to station (station 1> station 2> station 3). One reason for this may be the fact that station 1 is a Jetty and landing terminal for all boats transport from the city to the hinterlands of southern- Ijaw and beyond. As such lots of petroleum products are landed on the jetty both for fueling and domestic use. This is result is in disagreement with the findings of [11] who experienced a trendless spatial variation of TPH in study stations with evidently different potential of TPH generation. However, it may be difficult to predict and identify which station and sources may produce more TPH. [12] put it aptly by saying that the exact identification of TPH sources to the Niger Delta soils is not feasible due to the variety of processes contributing to the formation and preservation of TPH in soils of this area.

Also, in all stations throughout the study the result indicate that the absorption of TPH by macrophyte tissues show that root>stem>leaf. The lower concentration of TPH in leaf samples may have been caused due to phytodegradation or phyto transformation of petroleum hydrocarbons which was subjected the contaminants to the bioremedial processes occurring within the areal part of plant itself[8].Hydrophobic chemicals are generally not sufficiently soluble in water or are bound so

strongly to the surface of the roots and may not pass beyond the root's surface due to the high proportion of lipids present at the surface, so cannot be easily translocated into the plant [13]

The mean level of Bioaccumulation factor (BAF) of TPH in plants amples was found to be 1 and lower than 1.The lower BAF value of leaf samples shows uptake of hydrophilic compound of petroleum hydrocarbons by root and translocation to the leaf through vascular system. In general, chemicals that are highly water soluble are not sufficiently sorbed to roots or actively transported through plant membranes [14].

Finally, these results show the mean value of TPH in macrophyte samples and sediments does not exceeded the average global permissible limit in soil (1000mg/l) and also lower than the phytotoxic level in the plants (1000-12000 mg/l)[8]**.** It can be concluded that the River Nun at Amasoma Axis is only mildly polluted but there is reason to still monitor its water characteristic and promulgate land use ordinances to protect its fishery and entire ecosystem

REFERENCES

[1] Neff, J.M (1979) Polycyclic aromatic hydrocarbons in the aquatic environment. Applied Science Publ. Ltd., London. 262pp.

[2] Hellegraeff, G.M. (1995) Marine Phytoplankton community in the AustralianRegion.Current Status and future threats. In State of the Marine Environment Report for Australia.Technical Annex1.Department of Environment, Sports and Territories. Canberra.

[3] Keough, M.J; Butler, A.J. (1995) Temperate subtidal hard substrata. In State of the Marine Environment Report for Australia.Technical Annex 1.pp37-52. Ocean Rescue Programme. Department of the Environment, Sports and Territories. Canberra.

[4] Agbozu, I.E; Ekweozor, I.K.E; Opuene, K. (2007) Survey of heavy metals in the catfish Synodontisclarias. Int. J. Environ. Sci. Tech, 4 (1), 93-97.

[5] Pocock, S.J; Smith, M: Baghurst, P. (1994) Environmental lead and children's intelligence; A systematic renew of epidemiological evidence. Br. Med .J. 304, 1189 – 1197.

[6] Koller, K; Brown .T; Spurgeon, A; Levy, L. (2004) Recent development in low level exposure and intellectual impairment in children. Environ. Health perspective. 112 (9), 987 – 994.

[7] Alam, I. A; Sadiq, M (1993) metal concentration in Antarctic sediment samples collected during the Trans-Antarctica 1990 expedition. Marine pollution Bull. 26, 523 – 527.

[8] Lotfinasabas, S , Gunale, V.R, .Rajurkar, N.S (2013) Petroleum Hydrocarbons Pollution in Soil and its Bioaccumulation in mangrove species, *Avicennia marina* from Alibaug Mangrove Ecosystem, Maharashtra, India, International Journal of Advancements in Research & Technology, Volume 2, Issue2, February-2013

[9] Bower, H. J (1979) Heavy metals in sediments of foundary covers cold spring. New York; environ SciTechnol, 13, 683 – 689.

[10] Gbaruko, B C; Friday, O. U (2007) Bio accumulation of heavy metals in some fauna and flora Int. J. Environ Sci. Tech. 4 (2) 197–202

[11] Adeniji, A.O, Okoh, O.O, Okoh, A.I (2017) Petroleum Hydrocarbon Profile of water and sediment of Algoa Bay, Eastern Cape, South Africa. Int. Journ. ofEnv. Res. Pub. Health, 14, 1263.

[12] Anunobi, C. K (2005) The physico-chemical and heavy metal characteristics of Aba River in Aba, Abia State (M.phil Thesis, 100pp. rivers State University of Science and Technology, Port Harcourt).

[13] Siciliano S.D, Germida J.J (1998b) Mechanisms ofphytoremediation: biochemical and ecologicalinteractions between plants and bacteria. EnvironReview 6: 65-79.

[14] Schnoor JL, Licht LA, McCutcheon SC, Wolfe NL,Carreira LH (1995) Phytoremediation of organic andnutrient contaminants. Environ SciTechnol 29 (7):318-323.

Poultry manure application and fallow improves peanut production in a sandy soil under continuous cultivation

James Aipa[1], Patrick S. Michael[2]

[1]University of Natural Resources and Environment, Private Mail Bag, Kokopo, East New Britain Province, Papua New Guinea

[2]Department of Agriculture, PNG University of Technology, Lae, MP 411, Papua New Guinea.

Abstract— *To meet our food security demands, Papua New Guinea (PNG) needs to improve smallholder subsistence agriculture by promoting the production of cash crops that mature early and have a high market value. Peanut is a typical example of a cash crop which potentially has a high market value, but pod yields are low due to declinein soil quality. A field experiment was conducted under 4 different land use systems (LUS) to evaluate the effects of continuous peanut cultivation on peanut pod yield and on selected soil properties. Peanut pod yield declined significantly under the continuous peanut and peanut/corn rotation systems; while the poultry manure and land fallow systems significantly increased pod yield. Over the 3 cropping seasons, significant changes in organic carbon; extractable potassium and CEC in all cropping systems occurred, while changes in total N was significant in the peanut/corn rotation and poultry manure cropping systems only. No significant changes in bulk density; field capacity; electrical conductivity; soil pH and available phosphorus were observed in all the 4 LUS over the 3 cropping seasons. We suggest that adequate fallow periods of more than 1 year and poultry manures are applied to enhance soil quality and improve peanut productivity and/or sustain peanut production in marginal lands under continuous cultivation.*

Keywords— Continuous cultivation, land use systems, peanut pod yield, soil properties.

I. INTRODUCTION

In PNG, agriculture supports the livelihood of more than 85% of the people who live mostly in the rural areas. Food security is therefore often perceived to be food secure; however, the lack of basic services and rural infrastructure indicate that most of the people are poor [1] and live far below the poverty line [2, 3];at 38% [4]. To meet the demands for food and the Government's Vision 2050 become a food secure country; policies need to be targeted at improving smallholder subsistence agriculture in the rural areas and integrate domestic markets to improve food security [5]. The peanut (*A. hypogaea* L.), is a cash crop that has a potentially high economic value [6, 7]. Peanut is high in protein and carbohydrates, and is a good source of food for human and livestock. In addition, peanut is rich in minerals like copper, manganese, potassium, calcium, iron, magnesium, zinc and selenium.

Reports show that peanut is the second most important oilseed crop cultivated in most parts of the tropical, subtropical and warm temperate climate regions [8, 9]. In PNG, peanut is amongst the top five income generating cash crops [6]. Peanut is widely grown and marketed from the coastal lowlands to the mid–high altitude highlands of PNG, mainly in family owned subsistence farming systems. The crop has therefore become increasingly important as a cash crop and plays a significant role in family owned subsistence farming systems. Although the peanut has a high market potential, large-scale production in PNG is limited due to lack of high–yielding varieties, seed supply and suitable cost–effective farming practices [6].

The peanut, at the farm level has the ability to nourish soils, when grown in rotation or in combination with other food crops. As a result of rotation and/or intercropping, peanut has been implicated to increase yields of succeeding crops in rotation and/or component crops in intercrop systems. This is attributed to the activity of soil microbes that enhance productivity and quality of agricultural soils as a result of their influence on nutrient cycling, detoxification processes and soil aggregate stability, among other functions [10]. It has been reported that frequent use of peanut in crop rotation resulted in increase in soil–borne pathogens and relatively poor crop performance [11]. In continuous peanut cultivation systems, low peanut yields were associated with reductions in soil quality brought about by the changes in soil microbial community [12]. Continuous

cultivation of peanut also reduced bacteria species and quantity of bacteria and actinomycetes, lower the number of fungi species and increase mould quantity [13]. Similar effects have been reported in peanut by [14; 15] and in cucumber by [16]. One of the reasons for continuous cultivation in tropical regions is population pressure on land.

The global human population grows at an annual rate of 1.7% and it is projected to double at this rate in 40 years. The [17] reported that PNG's annual population growth rate is 2.3%. This has resulted in the decline in productive arable croplands under cultivation and farming communities have resorted to continued cultivation on existing croplands. The practice of continuous crop cultivation on the same piece of land has led to rapid nutrient mining and it is believed to be more severe in the tropical regions. In PNG and most parts of the tropics, shifting cultivation and bush fallow systems were practiced to restore soil fertility; however, fallow periods are shorter now due to increasing pressure on existing cropland by rapid increases in population. On a global scale, the degradation of the soil physical, chemical and biological properties is a major concern as 40% of agricultural land degradation is induced by anthropogenic processes [18]. Although the peanut industry is not fully developed in PNG, peanut can be used as an alternative cash crop in crop rotation and/or intercropping systems to improve soil quality.

This paper reports the results of a field experiment conducted under rain fed conditions in four (4) different land use systems focused particularly on continuous peanut cultivation over a period of three (3) consecutive cropping seasons. The objectives were two fold; evaluate (i) the effects of continuous peanut cultivation system compared to peanut/corn rotation, and (ii) poultry manure and fallow LUS on peanut pod yield and on selected soil properties.

II. MATERIALS AND METHODS

2.1 Experimental Site

The study was conducted between 2003 and 2004 at the agriculture model farm of PNG University of Technology, Lae in the Morobe Province. The farm ($6^{o}41$'S, $146^{o}98$'E) is located at an altitude of 65 m above sea level with a mean annual rainfall of up to 3,800 mm, which is fairly distributed throughout the year. Average daily temperature is 26.3^{o}C, with an average daily minimum of 22.9^{o}C and average daily maximum of 29.7^{o}C. Annual evaporation (US Class A pan) is 2,139 mm and rainfall exceeds evaporation in each month. The climate is classified as Af (Koppen) i.e. a tropical rainy climate that exceeds 60 mm rain in the driest month. The soil at the experimental site is well drained and derived from alluvial deposits. It is classified as a sandy, mixed isohyperthermic, Typic Tropofluents (US Soil Taxonomy) or EutricFluvisol (World Reference Base).

2.2 Experimental Treatments

The experiment was conducted with peanut (*A.hypogaea L.*) as the test crop. The field experimental design consisted of a replicated (n=4) randomized complete block design with 4 treatments (LUS), i.e. continuous peanut cultivation, crop rotation (corn), deep litter poultry manure (15 kg/replicate/25 m^2 plots) and natural fallow (planting to maturity of peanut as the test crop) for comparison. Chicken manure was applied at rate of 15kg/plot, which is equivalent to 6 tonnes per ha^{-1} by broadcasting over each plot and mixed into soil by raking before planting. In the continuous cultivation treatment, peanut was continuously cultivated for 3 consecutive cropping seasons at a spacing of 30 cm (within row) by 50 cm (between rows) giving a plant density of 187 plants per plot (25 m^2). In the rotation treatment, corn was planted during the second cropping season and in the fallow treatment, the plots were given a rest from planting to harvesting of peanut. In the chicken manure treatment, peanut was planted continuously for the 3 consecutive cropping seasons and 15 kg of chicken manure was applied to each plot measuring 5 m x 5 m (25 m^2).

2.3 Peanut Pod Yield Measurement

Each plot of peanut was harvested 110 to 115 days after actual field planting. For the measurement of peanut pod yield, plots of peanut for all the treatments were harvested on the same day to avoid discrepancies in pod weight and on soil properties. After harvesting, the peanut pods were thoroughly washed with the root stalks intact and sun-dried. The sun-dried peanut

pods were removed from the root stalks and pod yield was determined by measuring fresh weight of pods using a scale (Dillon type).

2.4 Measurement of Soil Properties

Soil samples for chemical analysis were collected from the experimental site before and after each cropping season and air-dried. The initial soil sample was collected before the experimental site was put to mechanical cultivation. Subsequent samples were taken after harvesting at the end of each cropping season to determine changes in soil properties. Particle size distribution was determined using the hydrometer method as described by [19]; bulk density using the soil core method, field capacity by taking moisture measurements of air-dried soil; electrical conductivity measurements by a standard dilution method of a 1:5 (w:v) using a conductivity meter and pH was measured with a 1:5 (w:v) ratio of soil to deionized water using a pH meter.

To determine soil chemical properties, air-dried soil samples were subjected to soil chemical analytical methods of [20] followed the procedures used by the National Analytical Laboratory (NAL), PNG University of Technology, PNG. Organic carbon was determined using the rapid wet oxidation method, in which soil samples were oxidized by a solution of 0.133 mol L^{-1} $K_2Cr_2O_7$ – 18.4 mol L^{-1} H_2SO_4 in an oil bath and excess $K_2Cr_2O_7$ was titrated with 0.2 mol L^{-1} $FeSO_4$, and thus organic matter content was obtained by multiplying the carbon value by a factor of 1.72. Total nitrogen was measured by the Semi-micro Kjeldahl method after soil samples were digested with $HClO_4$ and HF. Available phosphorus was extracted with a 0.5 mol L^{-1} $NaHCO_3$ solution and determined by molybdenum-blue colorimetry. Extractable potassium was extracted with 0.5 mol L^{-1} NH_4OAc (pH 8.5) and then determined by flame photometry. Cation exchange capacity was measured by a 0.01 mol L^{-1} $(AgTU)^+$ method and then determined by atomic absorption spectrometry (AAS).

2.5 Statistical Analysis

All basic statistical analyses were performed using MS Office XLSTAT and SPSS 14 (SPSS Inc. ILL. Canada). One-way analysis of variance (ANOVA) was performed followed by Tukey's Multiple Comparison Test to determine significance ($p = 0.05$) of mean differences between peanut pod yield and selected soil properties between the 4 LUS and the 3 cropping seasons.

III. RESULTS AND DISCUSSION

3.1 Peanut Pod Yield

Highest peanut pod yield was obtained from the continuous and manure systems during the first cropping season and the poultry manure and fallow systems during the third cropping season (Tables1). Peanut pod yields declined in the continuous and manure systems during the second cropping season. Yield decline from the continuous peanut system indicates the loss of soil quality resulting from continuous tillage. The decline in peanut pod yield from the poultry manure system shows that the applied manure rom the first cropping season did not affect peanut pod yield. In the rotation and fallow systems, peanut was rotated with corn and the plots were given a break in the second cropping season, respectively, thus no peanut pod yield data is provided. On an aggregate basis, peanut pod yield was higher in the poultry manure system than the continuous peanut, rotation and fallow systems (Table 1).

Peanut pod yield declined by 36% after the second cropping season and 16% after the third cropping season in the continuous peanut cropping system and 29% after the third cropping season in the crop rotation system (Table 1). The declines in peanut pod yield under these cropping systems could be attributed to declines in nutritional status of the soils shown by this study and studies by other researchers like [21; 22] and [23]. The continual application of chicken manure at 15 kg/plot led to a decline in peanut pod yield by 13% after the second cropping season but increased by 31% after the third cropping season (Table 1). In the fallow system, there was an increase in pod yield by 28% after the plots were fallowed for a period of 110 to 115 days during the second cropping season.

TABLE 1
PEANUT POD YIELD IN t ha^{-1} UNDER 4 DIFFERENT LAND USE SYSTEMS OVER 3 CROPPING SEASONS (2003–2004).

Cropping Season	Cropping systems				
	Continuous	Rotation	Manure	Fallow	Mean
1	3.54±0.03a	3.22±0.29a	3.66±0.08bc	3.01±0.12b	3.36a
2	2.60±0.22bc (36–)	–	3.25±0.13c (13–)	–	1.46b
3	2.25±0.11c (16–)	2.50±0.14b (29–)	4.25±0.11a (31+)	3.85±0.29a (28+)	3.23a
Mean	2.81bc	1.91d	3.72a	2.29cd	
LSD (0.05)	0.455	0.735	0.401	0.805	

All values are means of 4 replicates per cropping season for each cropping system and mean values within columns and rows followed by the same letter are not significantly different (p= 0.05). Numbers in parentheses () shows % decline (–) or increase (+) in peanut pod yield over the 3 cropping seasons

Peanut pod yield declined progressively in the continuous peanut LUS and peanut/corn rotation LUS (Table 1, Figure 1). In the poultry manure LUS, peanut pod yield declined after the second cropping season and increased after the third cropping season; while in the fallow system, pod yield increased after the plots were rested for 110 to 115 days. The application of deep litter poultry manure benefited the 3rd crop of peanut. The most likely reason is that nutrients from the applied poultry manure were made available to peanut during the 3rd cropping season and also due to improvement in soil quality in both the manure and fallow systems. Similar results were reported by[24; 25]. During the first and second cropping seasons, mean peanut pod yield between the 4 LUS's were not significantly different (*p= 0.05*); however, after the third cropping season, mean peanut pod yield were significantly different (*p= 0.05*).

In the continuous peanut LUS, peanut pod yield determined by one–way ANOVA showed significant differences between the 3 cropping seasons at F ((2, 9) = 84.4, *p= 0.000*). The Tukey post–hoc test revealed that peanut pod yield was significantly lower after taking cropping season 2 (2.60 ± 0.22 min, *p= 0.000*) and cropping season 3 (2.30 ± 0.11 min, *p= 0.037*) compared to cropping season 1 (3.54 ± 0.03 min). There was no significant difference observed between continuous peanut cropping seasons 2 and 3, *p= 0.037* (Table 1, Figure 1). Soil bulk density, pH and total N analysis showed consistent results and thus, the results of this study failed to justify the reasons for the declining peanut pod yield. The significant changes observed for extractable K and CEC suggests that the declining peanut pod yield in continuous peanut cropping systems was attributed to K and Ca interaction resulting from the higher values observed in this study. Similar results have been reported on declining peanut pod yield due to K and Ca interaction in other studies [9; 26].

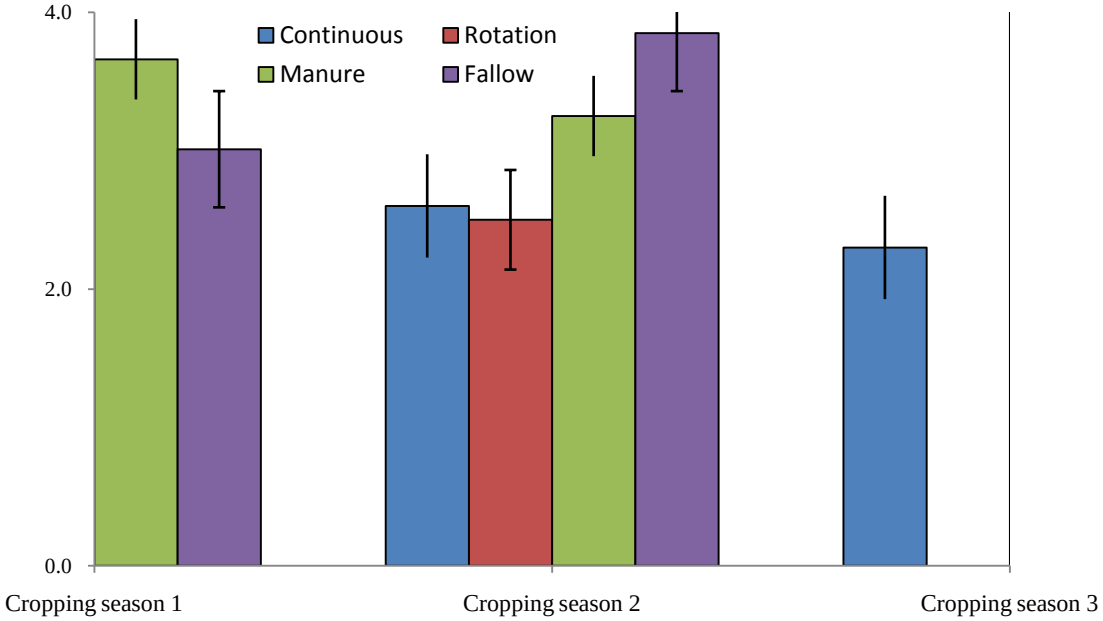


FIGURE 1. MEAN PEANUT POD YIELD OF THE DIFFERENT LAND USE SYSTEMS OVER 3 CROPPING SEASONS. ERROR BARS ARE MSE AND MSE FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT (*p= 0.05*).

The declining trend observed in peanut pod yield in the continuous peanut LUS conforms to the many well documented evidences of yield declines in continuous cropping systems, not only in peanut, but other crops as well. Yield decline in the rotation system after the third cropping season resulted from nutrient removal by corn during the rotation season (cropping season 2). The importance of a good rotation was evident in this study (data for corn not shown). The increase in peanut pod yield observed in the manure and fallow LUS's were attributed to residual effects from previous fertilization [27] and nutrient accumulation over the duration of the applied poultry manure and short fallow periods. Based on our results, there is sufficient evidence to say that the progressive declines in peanut pod yield in the continuous peanut LUS could be a direct result of the antagonistic effects due to interactions between different nutrients, particularly K and Ca interactions.

3.2 Soil properties

In the continuous peanut cropping system, bulk density, pH and total N remained constant, while field capacity, electrical conductivity, organic carbon, available P, extractable K and cation exchange capacity declined over the cropping seasons, compared to results from a natural control plot from the experimental study. Bulk density in the continuous peanut LUS was high compared to the natural control (NC) that showed a declining trend. Soil pH also remained constant over the cropping seasons, which does not support our assumption that the declining peanut pod yield was attributed to an increase in soil acidity. There was no change in total N levels, while available P declined by 39% after the third cropping season. ANOVA showed no significant ($p= 0.05$) changes in bulk density, FMHC, EC, pH, total N and available P. We observed significant ($p= 0.05$) changes in OC, extractable K and CEC activity over the 3 cropping seasons (Table2).

Peanut was rotated with corn in the crop rotation system in the second cropping season. Bulk density and pH remained constant, when compared to the natural control. FMHC and available P declined and increased again after the third cropping season; while EC and extractable K declined. With the rotation system, there was an increase in organic C, total N and CEC, compared to the continuous peanut, manure and fallow systems. Similar results of these soil chemical properties were observed in the natural control. ANOVA showed no significant ($p= 0.05$) changes in bulk density, FMHC, EC, pH and available P, but there were significant ($p= 0.05$) changes in OC, total N, extractable K and CEC. The changes in OC, total and CEC were positive and for extractable K was negative (Table, 2).

Manure was applied at a rate of 15 kg per plot (25 m^2) and peanut was planted continuously over the 3 cropping seasons. In the manure system, bulk density and pH declined after the first crop and remained constant thereafter, a phenomenon that was similar to the other cropping systems when compared to the results of the natural control plot. Manure positively affected FMHC and CEC by increasing moisture retention and CEC. Organic C declined after the second crop but increased after the third crop. The continual application of deep litter poultry manure resulted in an increase in organic C compared to the other cropping systems. Total N, available P and extractable K, all declined progressively over the 3 cropping seasons. The changes observed were not significant ($p=0.05$) for bulk density, FMHC, EC, pH and available P. With the continual application of manure, signification ($p=0.05$) changes in OC, total N, extractable K and CEC (Table2).

In the fallow treatment, plots were fallowed for 110 to 115 days, which was the length of the peanut growing season. There were no changes in soil bulk density and pH over the 3 cropping seasons. FMHC, total N and CEC saw a slight decline after the first crop, but increased again after the third crop. EC, organic C and available P declined after a fallow, while extractable K increased after the land was fallowed. Our results showed that the changes in bulk density, FMHC, EC, total N and available P were not significant ($p=0.05$). After the land was given a break, significant ($p=0.05$) changes were observed in organic C, extractable K and CEC, for example, extractable K increased by 41% (Table 2). The higher peanut pod yield attained after the fallow period shows the importance of land fallows, in this case a significant increase in peanut pod yield (Table, 1) compared to the continuous peanut cropping system.

TABLE 2
INITIAL STATUS AND CHANGES IN SELECTED SOIL PROPERTIES OF DIFFERENT LAND USE SYSTEMS OVER 3 CROPPING SEASONS (2003–2004)

Land use system	Cropping season	Soil properties								
		D_b	FMHC	EC	pH	OC	Total N	P_{av}	K_{ext}	CEC
		(g/cm^3)	(%)	($dS\ m^{-1}$)	(1:5)	(%)	(%)	(mg/kg)		(mEq./100g)
Continuous	Initial	1.4	26	5.6	6	1.4	0.24	117	373	26
	1	1.4	27	5.3	5.9	1.5	0.14	166	470	30
	2	1.4	27	4.5	5.9	1.4	0.14	111	430	26
	3	1.4	26	4.5	5.9	1.4	0.14	101	410	26
	Mean	1.4ns	27ns	4.8ns	5.9ns	1.43*	0.14ns	126ns	437*	27*
	LSD (0.05)	0.13	0.48	0.12	0.01	0.04	0.13	5.54	15.35	65.1
Rotation	Initial	1.4	26	5.6	6	1.4	0.24	117	373	26
	1	1.4	25	5.4	5.9	1.3	0.14	140	490	27
	2	1.4	26	4.6	5.9	1.4	0.17	137	480	29
	3	1.4	24	4.6	5.9	1.4	0.2	138	478	29
	Mean	1.4ns	25ns	4.9ns	5.9ns	1.37*	0.17*	138ns	483*	28.3*
	LSD (0.05)	0.13	0.46	0.13	0.01	0.04	0.13	5.54	15.35	64.7
Manure	Initial	1.4	26	5.6	6	1.4	0.24	117	373	26
	1	1.4	24	5.1	6	1.7	0.16	144	510	28
	2	1.4	25	4.5	5.9	1.4	0.14	119	410	30
	3	1.4	27	4.5	5.9	1.7	0.12	120	400	31
	Mean	1.4ns	25ns	4.7ns	5.9ns	1.6*	0.14*	128ns	440*	30*
	LSD (0.05)	0.13	0.44	0.12	0.01	0.04	0.13	5.35	15.65	64.51
Fallow	Initial	1.4	26	5.6	6	1.4	0.24	117	373	26
	1	1.3	28	5.1	5.9	1.4	0.14	159	520	31
	2	1.3	26	4.2	5.9	1.3	0.13	145	530	29
	3	1.3	28	4.2	5.9	1.3	0.14	144	560	30
	Mean	1.3ns	27ns	4.5ns	5.9ns	1.3*	0.14ns	149ns	522*	30*
	LSD (0.05)	0.13	0.48	0.12	0.01	0.04	0.13	5.54	15.15	64.1
Natural Control	Initial	1.4	26	5.6	6	1.4	0.24	117	373	26
	1	1.4	27	6.3	5.9	2	0.2	111	430	30
	2	1.2	24	8	5.9	2	0.21	120	730	29
	3	1.2	28	8.4	5.9	2.1	0.27	124	774	30
	Mean	1.3ns	26ns	7.6*	5.9ns	2.0ns	0.23*	118*	645*	30*
	LSD (0.05)	0.13	0.48	0.13	0.01	0.04	0.13	5.64	15.85	65.4

Mean values with an asterisk (*) within rows indicate significant changes (ANOVA, p = 0.05); ns = not significant. D_b= bulk density, FMHC = field moisture holding capacity, EC = electrical conductivity, pH = soil pH, OC = organic carbon, Total N = total nitrogen, P_{av} = Available phosphorus, K_{ext} = extractable potassium, CEC = cation exchange capacity

In a natural control plot, bulk density declined as expected. This is similar to results of no tillage experiments by [28] and by [29]. FMHC and CEC declined after the second crop and increased after the third. No soil pH changes were observed. EC, organic C, total N, available P and extractable K all increased as expected. Changes in bulk density, FMHC, pH and organic C were not significant (*p=0.05*). On the contrary, changes in EC, total N, available P, extractable K and CEC were significant (*p=0.05*). The results of the selected soil properties evaluated in this study confirm that land under crop cultivation loses its quality especially under continuous cropping. It is therefore suggested that both organic and inorganic materials be supplied; good crop rotations practiced and the land given a sufficient break to improve soil quality to sustain peanut pod yield.

IV. CONCLUSION

We evaluated peanut growth responses under the following land use systems: continuous peanut, peanut/corn rotation, continuous peanut with poultry manure and fallow systems in a sandy loam soil from 2003 to 2004. Our evaluation of peanut pod yield and selected soil properties showed the poultry manure and fallow systems were more favourable for peanut cultivation than the continuous peanut and peanut/corn rotation systems. The application of poultry manure at 15 kg per plot (25 m^2) is equivalent to 6t/ha and a break period of 110 to 115 days produced better peanut pod yield and had favourable effects on soil properties. Although the study results showed that the nutritional status of the soil was adequate to sustain peanut growth and productivity, soil pH and soil water retention capacity were factors that could hinder peanut production. The peanut can generate high economic returns for smallholder farmers; therefore to improve soil quality for better peanut production, poultry manure and fallows are recommended.

ACKNOWLEDGEMENTS

The study was funded by the PNG University of Technology through its Graduate Assistant Scheme at the Department of Agriculture. The study would not have been successful without the efforts of Mrs. Patricia Sariman of the Career Development Office, the supervisor of the first author Dr. Mohammed Muneer and the faculties, technical staff and students of the Department of Agriculture. We also acknowledge the family of the first author, especially wife Kusi Aipa, for their patience and love during the study.

REFERENCES

[1] Cammack D (2009) Chronic Poverty in Papua New Guinea: Background Paper for the Chronic Poverty Report 2008–09. http://www.chronicpoverty.org/uploads/publication_files/.

[2] Chandy L (2009) Linking Growth and Poverty Reduction in Papua New Guinea. Lowy Institute for International Policy, September 2009 Analysis.

[3] Gibson J, Olivia S (2008) 'Attacking Poverty in Papua New Guinea, But For How Long?', *Pacific Economic Bulletin* 17(2): 63-71 (2002)). Accessed on 30 May 2012.

[4] FAO (2011) Countries: Papua New Guinea, Food Security in Papua New Guinea. www.fao.org/countries/55528/en/png/.

[5] Chand S, Yala C (2011) Policies for improving food security. Keynote paper presented at the *Food Security Policy Conference* on the theme "*Macroeconomic Considerations, Policy Issues and Regulatory Mechanisms*" held at the National Research Institute, Port Moresby from 6-8 September 2011. Accessed on 30 May 2012.

[6] ACIAR (2006) Improving yield and economic viability of peanut production in Papua New Guinea and Australia using integrated management and modelling approaches. http://aciar.gov.au/project/ASEM/2001/055.

[7] ACIAR (2008) Growing peanuts in Papua New Guinea: a best management practice manual. ACIAR Monograph No.134, 77 pp. Accessed on 25 May 2012.

[8] Acosta–Martínez A, Rowland D, Sorensen RB, Yeater KM (2008) Microbial community structure and functionality under peanut–based cropping systems in a sandy soil. BiolFertil Soils 44:681–692. Accessed 25 May 2012.

[9] Gashti AH, Vishekaei MNS, Hosseinzadeh MH (2012) Effect of Potassium and Calcium Application on Yield, Yield Components and Qualitative Characteristics of Peanut (*Arachishypogaea*L.) in Guilan Province, Iran. World Applied Sciences Journal 16(4): 540–546. Accessed on 30 May 2012.

[10] Marcela SM, Olga SC, Marcelo AS, Serita DF, Augusto FG, Jay LG(2011). Multivariate approach to characterizing soil microbial communities in pristine and agricultural sites in Northwest Argentina.Appl. Soil Ecol., 47(3): 176-183.

[11] Bell M, Harch G, Tatnell J, Middleton K (2002) The impact of crop rotation on peanut productivity in rainfed cropping systems. Australian Society of Agronomy, Agency for Food and FibreScience, Farming Systems Institute, QDPI, PO Box 23 Kingaroy 4610.Qld. [Pdf online]. Accessed on 30 May 2012.

[12] Xu RF, Wang XL (2003) Relation of microbial population dynamics and nutrient in soil of continuous cropping with peanut. J. Peanut Sci., (in Chinese) 32(2): 19–24.

[13] Acosta–Martínez V, Mikha MM, Vigil MF (2007) Microbial communities and enzyme activities in soils Ander alternative crop rotations compared to wheat–fallow for the Central Great Plains. Appl Soil Ecol 37:41–52.Accessed 25 May 2012.

[14] Feng HS, Wan SB, Zuo XQ, Cheng B (1999) Changes of microbial community in soil and rhizosphere as affected by continuous cropping and their relation to peanut yield. Peanut Sci. Tech. (in Chinese) (Supplement), 277–283.

[15] Zhang SS, Fen HS, Wan SB, Sui QW, Zuo XQ (1992) Effect of continuous cropping years on peanut growing. J. Peanut Sci. Tech. (in Chinese) 2: 21–23

[16] Hu YS, Liu YF, Wu K, Dou HJ, Jia XC (2006) Variation of microbial community structure in relation to successive cucumber cropping soil. Chin. J. Soil Sci., 37(1): 126–129.

[17] World Bank (2011) World Development Indicators. Population growth rate, Papua New Guinea. www.indexmundi.com/papua_new_guinea/population_growth_rate.

[18] Scherr, S. J. and Yadav, S. 1997. Land degradation in the developing world: issues and policy options for 2020. International Food Policy Research Institute, 2020 Brief 44, Washington DC, USA.

[19] Day, P. R. (1953). Experimental confirmation of the hydrometer theory. Soil Sci. 64, 181-186.

[20] Walkley, A. and Black, I. A. 1964. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 1934;37:29–38.

[21] Brady NC, Weil RR (1999) The Nature and Properties of Soil (12th Edition). Prentice Hall, Inc., Pearson Education, Upper Saddle River, New Jersey, 07458.

[22] Tisdale S, Havlin JL, Beaton JD, Nelson WL (1999) Soil Fertility and Fertilisers: An Introduction to Nutrient Management. (6th Edition). Prentice Hall, Inc., New Jersey.

[23] Sanchez PA (1976) Properties and Management of Soils in the Tropics. John Wiley and Sons Limited. New York.

[24] Agboola AA (1982) Organic manuring and green manuring in tropical agricultural systems. Proc. 12th International Congress of Soil Science, New Delhi, India, 8–16th February, 1982. pp: 198.

[25] Singh L, Verma RNS, Lohia SS (1980) Effect of continuous application of farmyard manure and inorganic fertilizers on some chemical properties of a semi-arid soil of India. *Journal of Indian Society of Soil Science*, 28: 170–172.

[26] Pettigrew, WT (2008) Potassium influences on yield and quality production for maize, wheat, soybean and cotton. Physiologia Plantarum 133: 670–681.

[27] Agri–Briefs (1998) Agronomic News Items, Peanut Nutrition – A Best Management Practice for Top–Profit Peanut Production. Potash & Phosphate Institute. Spring 1998, No: 5.

[28] Agboola AA (1981) The Effect of different soil tillage and management practices on the physical and chemical properties of soil and maize yield in a rainforest zone of western Nigeria. *Agronomy Journal*.73(2): 247–251.

[29] Lal, R (1976) No tillage effects on soil properties under different crops in western Nigeria. *Soil Science Society of American Journal*. 40(6): 762–768.

AD Publications
Sector-3, MP Nagar, Rajasthan, India
www.adpublications.org, www.ijoear.com, info@ijoear.com