IJOEAR

International Journal of Environmental & Agriculture Research

www.ijoear.com

ISSN
2454-1850

Volume-6, Issue-9, September 2020

Preface

We would like to present, with great pleasure, the inaugural volume-6, Issue-9, September 2020, 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:

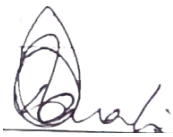
Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment,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



Dr. Bhagawan Bharali
(Chief 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. Goswami Tridib Kumar

Presently working as a Professor in IIT Kharagpur from year 2007, He Received PhD degree from IIT Kharagpur in the year of 1987.

Prof. Khalil Cherifi

Professor in Department of Biology at Faculty of Sciences, Agadir, Morocco.

Dr. Josiah Chidiebere Okonkwo

PhD Animal Science/ Biotech (DELSU), PGD Biotechnology (Hebrew University of Jerusalem Senior Lecturer, Department of Animal Science and Technology, Faculty of Agriculture, Nau, AWKA.

Prof. Özhan ŞİMŞEK

Agriculture Faculty, Department of Horticulture, Çukurova University, Adana, 01330 Turkey.

Dr. Anka Ozana Čavlović

Working as Professor in the department of Faculty of Forestry, University of Zagreb, Svetošimunska 25, Zagreb.

Dr. Rakesh Singh

Professor in Department of Agricultural Economics, Institute of Agricultural Sciences, Banaras Hindu University, Also Vice President of Indian Society of Agricultural Economics, Mumbai.

Dr. Sunil Wimalawansa

MD, PhD, MBA, DSc, is a former university professor, Professor of Medicine, Chief of Endocrinology, Metabolism & Nutrition, expert in endocrinology; osteoporosis and metabolic bone disease, vitamin D, and nutrition.

Dr. Ajeet singh Nain

Working as Professor in GBPUA&T, Pantnagar-263145, US Nagar, UK, India.

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 overseas Associateship for Post-Doc at WSU, USA – April, 2012 to October, 2012.

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.

Dr. Mahendra Singh Pal

Presently working as Professor in the dept. of Agronomy in G. B. Pant University o Agriculture & Technology, Pantnagar-263145 (Uttarakhand).

Dr. Vijay A. Patil

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

Prof. Salil Kumar Tewari

Presently working as Professor in College of Agriculture and Joint Director, Agroforestry Research Centre (AFRC) / Program Coordinator in G.B. Pant University of Agric. & Tech., Pantnagar - 263 145, Uttarakhand (INDIA).

Dr. S. K. Jain

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

Dr. Deshmukh Amol Jagannath

Presently working as Assistant Professor in Dept. of Plant Pathology, College of Agriculture polytechnic, NAU, Waghai.

Mr. Anil Kumar

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

Mr. Jiban Shrestha

Scientist (Plant Breeding & Genetics)

Presently working as Scientist (Plant Breeding and Genetics) at National Maize Research Programme (NMRP), Rampur, Chitwan under Nepal Agricultural Research Council (NARC), Singhdarbar Plaza, Kathmandu, Nepal.

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.

Mr. Isaac Newton ATIVOR

MPhil. in Entomology, from University of Ghana.

He has extensive knowledge in tree fruit orchard pest management to evaluate insecticides and other control strategies such as use of pheromone traps and biological control to manage insect pests of horticultural crops. He has knowledge in agronomy, plant pathology and other areas in Agriculture which I can use to support any research from production to marketing.

Mr. Muhammad Farooq

Mphil (Food Technology) Gold Medalist

PhD Scholar, Food Science and Biological Engineering, Jiangsu University in China

Production Manager Zeal Food & Beverages Industry Aladand Road, Amandara Batkhela Malakand Agency KP. He developed the skills for Preparation of different fruit pulp preserve Orange, Peach, Guava, Strawberry, and orange squash, orange marmalade.

Table of Contents

Perception of effect of climate change and adaptation strategies of beekeepers of Welmera district, Ethiopia

Lalisa Wendimu Biyena

Department of Socio-economics, Agricultural Extension and Gender Research, Oromia Agricultural Research Institute, Ethiopia

Abstract— *This study identifies factors affecting smallholder beekeepers' decisions to choose strategies to adapt to climate change in Welmera District, Oromia regional state, Ethiopia. Accordingly, quantitative data analysis and a multinomial logit model was used to identify perception of effects of climate change and adaptation strategies, and factors influencing beekeepers' choice of adaptation strategies to climate change, respectively. Results signified that skip honey harvesting, additional feeding, bee hive shade and improved bee forage planting are the dominant adaptation strategies that smallholder beekeepers used to limit the negative impact of climate change. The result from the multinomial logit analysis showed that age, education, family size, farm size, income, perception of effects of climate change, membership to beekeeping group, and access to beekeeping extension contact were significance factors influencing adaptation strategies of beekeepers. This would be a catalyst in developing and implementing appropriate as well as viable adaptation strategies in beekeeping practices context.*

Keywords— Adaptation strategies, beekeepers, climate change, MNL, Welmera.

I. INTRODUCTION

Climate change affects agriculture (higher temperature, reduced rainfall, etc.) [1].With a relatively low capacity to absorb the shocks of such events, Ethiopia's economy is at risk of losing out on the gains that the country has made through its impressive economic growth in recent years [2].

Plant-pollinator interactions are important for food production, and maintaining biodiversity ([3], [4]). Honeybees are the most effective pollinators of flowering plants [5].

Beekeeping is a long standing practice in Ethiopia and it accounts for 1.3% of agricultural GDP [6].

Climate change is reported to influence honeybees through its effects on their resource bases [7]. This impact of climate change on honey yields is poorly understood; this lack of understanding of the effects of climate change on honey yields is prevalent in developing regions [8].

Climate change adaptation is crucial [9] in response to damage due to climate change. Because of the huge contribution of honey production to beekeeper farmers' economy and its high susceptibility to climate change, it is important to study beekeepers' adaptation strategies to overcome the anticipated adverse impacts of climate change.

Various studies on adaptation strategies to climate change and determinants of farmer's adaption decision in Ethiopia ([10], [11]) have significant limitations. It emphasizes crop production and disregards the adaptation measures to climate change on beekeeping activity and its links with crop production. This oversight may underestimate the factors affecting beekeepers with regard to climate change. Next, most of the studies are conducted in lowland and rift valley areas of Ethiopian and overlook highland area. However, climate change expected to have an influence on both moisture-sufficient highlands and the drought-prone areas. Once more, none of the works focused beekeeping related climate change in the study area. Therefore, the study focused on i) to examine beekeepers' perception of effects of climate change on honey production ii) to identify beekeepers' adaptation strategies in response to climate change, and assess the factors influencing beekeepers' choice of climate change adaptation strategies.

II. METHODOLOGY

2.1 Description of the study area

The study was conducted in Welmera district of Oromia Special Zone, Ethiopia. The district is 29 km away in West of Addis Ababa at 9002N latitude and 38034E and altitude ranges from 2000-3380 meter above sea level. It has annual rainfall and temperature ranging from 334-1350mm and 0.1C°-27C° respectively, and with mean annual rainfall of 1067mm and mean temperature of 18 C°. Crop-livestock mixed farming system characterizes agriculture in the district. The district is potential in honey production.

2.2 Sampling method and data collection method

Study population for the study was farmer beekeepers own any types of hive of the study area. The district was selected purposively. Primary data was collected on 164 smallholder beekeeper household heads using probability proportional sampling techniques to the size of the population in the selected seven kebeles, andfocus group discussants. The sampling size for the households' survey was determined using the rule $N \geq 50 + 8m$ [12] in order to assure that the econometric model could be estimated with sufficient degrees of freedom, where N = sample size, and m = number of explanatory variables.

The focus group discussions for this study were held with separate groups of elders in both Dega and Woynadega agro-ecology comprising 5–10 individuals per group. The sessions were moderated by the researcher using a checklist including climate change impact in the area, and what factors influenced beekeepers' adaptation decisions. Secondary data which support primary data were collected from different sources like research articles, internet and concerned offices.

2.3 Methods of data analysis

The qualitative analysis used interpretation by narrating the response of the respondents and focus group discussants. The quantitative data analysis used descriptive statistics to summarize beekeepers' perceived effects of climate change on honey production and existing adaptation strategies to climate change effects, and Multinomial logit model (MNL) used to determine factors affecting beekeepers' adaptation decision using STATA version 13.

2.4 Econometric data analysis

Either multinomial logit or multinomial probit regression model used when there is a dependent variable with more than two alternatives (i.e. unordered qualitative or polytomous variables). Both of them estimate the effect of explanatory variables on dependent variable involving multiple choices with unordered response categories [13].

In this study a MNL was employed as it's easy to compute and also MNL analysis exhibits a superior ability to predict adaptation strategies and picking up the differences between adaptation strategies to climate change employed by households. This model makes it possible to analyze factors influencing beekeepers' choices of climate change adaptation strategies in the context of multiple choices.

Following Green [13] the MNL model for a multiple choice problem is specified as follows:

$$pij = \frac{e^{Xi\beta I}}{\sum_{j=1}^{j=5} e^{xi\beta j}}; j = 1 \dots \dots .5 \quad (1)$$

Where pij = is the probability of beekeeper's choice of adaptation strategies from category j), Xi= is predictors of response probabilities; e is the natural base of logarithms; and βj is the parameters to be estimated by maximum likelihood estimator (MLE).The estimated equations provide a set of probabilities for the j + 1 choice for a decision maker with Xi characteristics. For identification of the model, we need to conveniently normalize by assuming β=0 [13]. Therefore, the probabilities are given by:

$$Prob.\left(yi = \frac{j}{xi}\right) = pij = \frac{e^{xi\beta j}}{\sum_{j=2}^{j=j} e^{xi\beta j}}, for\ j > 1 \quad (2)$$

$$Prob.\left(yi = \frac{j}{xi}\right) = pi1 = \frac{1}{1+\frac{e^{xi\beta j}}{\sum_{j=2}^{j=j} e^{xi\beta j}}} \quad (3)$$

The marginal effects (δij) of the characteristics on the probabilities are specified as

$$\delta ij = \frac{\partial ij}{\partial x1}\rho ij[\beta j - \sum_{j=0}^{j} \rho ij\beta j] = \rho ij[\beta j - \beta^{-}] \quad (4)$$

Before running the model, the problem of multicollinearity among the continuous and dummy variables was detected. The variance inflation factor (VIF) was used for continuous predictor variable in the study. A VIF value greater than 10 is used as a signal for existence of severe multicollinearity [14]. For dummy variables if the values of contingency coefficient (CC) is greater than 0.75, the variable is said to be collinear. No multicollinearity occurred at all.

2.5 The summary of statistics for explanatory variables

Based on the review of adaptation literatures, 13 possible explanatory variables were considered in this study and examined for their effect in beekeeper's adoption decision of an adaptation strategy to climate change (Table 1).

III. RESULTS AND DISCUSSION

3.1 Beekeepers' perception of effect of climate change on honey production

Respondents were asked to indicate their response whether or not climate change had a negative effect on their honey production. The majority (70.12%) perceived climate affects their honey production negatively whereas 29.88% were not (Table 2). Table 2 further shows that, 22.56% and 21.34% of the respondents from Dega agro ecological zone had perceive effects of change in climate and taken adaptation measures to climate change respectively, while the remaining 19.51% and 20.73% did not. In the same way 47.56% and 46.34% the respondents from Woynadega agro ecological zone had already perceived effects of climate change and taken adaptation measures to climate change, respectively. However, as compared to the beekeepers between Dega and woynadega agro-ecological zones, the respondents who perceived and take adaptation measures from Woynadega agro-ecological zone are relatively higher. This is may be due to the fact that the intensity of climate related problems gets higher and higher as one goes from Dega to woynadega. Therefore, this proportion could also be an indicator of where the climate related problems is a little bit more sever. However, there are about 10.37% and 11.59% of the respondents did not yet perceive and taken adaptation measures to climate change from woynadega agro-ecological zone as compare to dega agro-ecological zone.

They perceived effects of climate change in beekeeping in terms of declining availability of bee forages; diminish honey yields; occurrence of bee colony absconding; and high temperature in the area. In this study, the weighted average index (WAI) was used to rank the effects of change in climate on honey production. Respondents were asked to score the effects of climate change based on a 0-2 Likert scale (i.e. in terms of 'high', 'moderate' and 'low'). Beekeepers generally perceive effects of climate change in terms of high, moderate and low. As per the past experiences, the rate of shortage of honey bee forages due to climate effect was ranked first by respondents (WAI=1.93) (Table 3). According to the interviewed beekeepers, this problem is directly related with deforestation of forest coverage from time to time for expansion of agricultural lands and various purposes which cause shortage of bee forage. When bee forage is exhausted, honeybee colonies suspended the capacity to rear brood; often provoking rapid population decline and resulted colonies absconding that may cause honey lose [15]. Moreover, slight diminish in honey yield due to adverse effects of climate change were ranked second (WAI=1.7). This may be happen due to simply a shortage of nectar-producing flowers because honeybee forage determines the amount of honey yield obtained provided that other factors are suitable for honey production. Honey bee colony absconding and high temperature were ranked third and fourth, respectively (WAI=0.93 and WAI=0.86) during study year. Bee colony loses can affect directly honey production and income of beekeeper. Among many, lack of food is major cause of bee colony absconding [16]. If the beehive temperature becomes too high then foragers will be busy collecting water, to reduce the nest temperature, rather than nectar or pollen. This will in turn affect honey production negatively. Group discussants also associated the impact of climate change with reductions in honey production and considered such reductions as a salient risk posed to their beekeeping activity.

TABLE 1
EXPLANATORY VARIABLES INCLUDED IN THE ANALYSIS

Variable	Description	Expected sign
Agro-ecology	Dummy takes the value of 1 if Woynadega and 0 otherwise	+
Sex	Dummy takes the value of 1 if male and 0 otherwise	+
Age	Continuous	+
Education	Dummy takes the value of 1 if literate and 0 otherwise	+
Family size	Continuous	+
Farm size in hectares	Continuous	±
Livestock	Continuous	+
Total income	Continuous	±
Perception	Dummy takes the value of 1 if perceived and 0 otherwise	+
Number of bee colony	Continuous	+
Membership	Dummy takes the value of 1 if a member and 0 otherwise	+
Extension contact	Dummy takes the value 1 if have access and 0 otherwise	+
Credit	Dummy takes the value 1 if have access and 0 otherwise	+

TABLE 2
PERCEPTION OF EFFECT OF CLIMATE CHANGE AND ADAPTATION STRATEGY ACROSS AGRO-ECOLOGY

Agro-ecology	Perception of effects of climate change (%)		Adaptation by per cent	
	Yes	No	Yes	No
Dega	22.56	19.51	21.34	20.73
Woynadega	47.56	10.37	46.34	11.59
Total	70.12	29.88	67.68	32.32

TABLE 3
RANK EFFECT OF CLIMATE CHANGE BY BEEKEEPERS

Variables	Ranking of occurrence			WAI	Rank
	High (2)	Moderate (1)	Low (0)		
Lack of bee forage	107	8	0	1.93	1
Reduced honey yield	77	35	3	1.7	2
Colony absconding	21	65	29	0.93	3
High temperature	18	63	34	0.86	4

3.2 Beekeeping adaptation strategies to climate change

In this survey, beekeepers were asked what adaptation strategies they have typically used in order to adapt to the negative impact of climate variability and changes in honey production. As a result, adaptation strategies used by beekeepers were

jump honey harvesting (21.95%), additional feeding (20.73%), bee hive-shading (17.07%), and bee forage planting (7.93%) in a declining proportion (Table 4). In general, 67.68% of beekeepers in the district had actually adapted to climate change compared to 32.32 % who do not adopt any of adaptation strategy. Jump honey harvesting, additional feeding and bee hive shade are indigenous practices while using improved bee forage is introduced development intervention to increase honey production.

The use of jump harvesting of honey as an adaptation strategies to climate change effects is to save honey to perpetuate strong colonies for next honey flow season as only strong colony survive compared to weak colony which is more vulnerable to any shocks of climate variability and change. According to beekeepers opinion taking honey during lack of sufficient pollen and nectar would dramatically weaken bee colony and expose bee colony to threats (absconding, starvation, and pest attack).

In the same way, interviewed beekeepers supplement their bee colony with additional feeds in the absence of bee forage as adaptation strategy to climate change. Similar studies also reported that about 90.7% of the beekeepers seem aware of supplying additional feeding for their bee colonies [17]. Some of interviewed beekeepers were preferred shade for beehive to reduce direct sun light radiation effect on bee activities. Also some beekeepers who perceived declining state of indigenous bee forages in their area started use of improved bee forage using micro-irrigation.

TABLE 4
ADAPTATION STRATEGIES BY BEEKEEPERS

Adaptation strategies	Per cent
Additional feeding	20.73
Bee forage planting	7.93
Beehive shade	17.07
Skip harvest	21.95
Not adapted	32.32
Total	100

3.3 Determinants of beekeepers' choice of adaptation strategies

Farmers' adoption behavior, especially in low-income countries, is influenced by a complex set of socio-economic, demographic, institutional and biophysical factors ([18], [19]). Statistically influential determinants are factors on which efforts should be exerted to enhance farm-level beekeeping adaptations to climate change and variability in the study area.

The result of MNL model showed how factors that influence beekeepers' choice of adaptation strategies in the study area. Table 5 represented the results of parameter estimates of MNL regression model with some significant levels of the parameters estimates. The likelihood ratio statistics as indicated by ch2 statistics (LR chi-square (52) = 2241.05 are highly significant $P < 0.0000$), suggesting the model has a strong explanatory power.

As indicated earlier, the parameter estimates of the MNL model provide only the direction of the effect of the independent variables on the dependent (response) variable: Estimates do not represent actual magnitude of change or probabilities. Thus, the marginal effects from the MNL, which measure the expected change in probability of a particular choice being made with respect to a unit change in an independent variable, are reported and discussed (Table 6). In all cases the estimated coefficients should be compared with the base category of no adaptation.

3.3.1 Age of the respondent

A one year increase in age of the household head, the likelihood of beekeepers' using planting of honey bee forage, jump honey harvesting and bee hive shade as adaptation strategy increases by 56.2%, 89.1% and 69.9% at 1%, 1% and 1% significance level respectively, holding other variable constant (Table 6). This means that the likelihood of taking up climate adoption strategies was higher among older beekeepers. Because as the age of the household head increases, the person is expected to acquire more experience in climate conditions and that helps take action to combat climate change.

3.3.2 Education of the respondent

As the household heads get access to education, the likelihood of using adaptation strategy additional feeding and bee hive shade increases by 29.8% and 74.5% at 5% and 1% level of significance respectively, holding the value of other variables constant (Table 6). This hints that the educated households are more sensitive for managing their bee colony by providing supplementary feeding, and reduce hot to reduce problems of effects of change in climate.

3.3.3 Family size of the respondent

MNL models show a one unit increase in the family size, the likelihood of beekeepers use improved bee forage as adaptation strategies increase by 23.3% at a significance level of 1% keeping other variables constant (Table 6).

TABLE 5
PARAMETER ESTIMATES OF MNL CLIMATE ADAPTATION MODEL

Variable	Additional feeding	bee forage	Skip harvesting	Bee hive shade
	Coef. P-value	Coef. P-value	Coef. P-value	Coef. P-value
Agroecology	1.733 0.190	1.085 0.526	1.603 0.236	1.396 0.284
Sex	0.0467*** 0.007	1.334 0.488	2.145 0.177	0.446 0.758
Age	0.188 0.415	0.545 0.335	0.340 0.151	0.228 0.102
Education	1.571 0.407	1.384 0.494	0.322 0.139	1.425 0.452
Family	0.412 0.313	0.035 0.155	0.179** 0.036	0.129 0.755
Farm size	0 .785** 0.016	-0.596** 0.023	0.148 0.115	-2.655 0.773
Livestock	0.934** 0.024	-0.1141** 0.011	0.004 0.706	0.6548 0.107
Totincome	-0.0004** 0.011	-0.002** 0.022	0.560 0.193	0.037** 0.010
Perception	0.331*** 0.001	0.019*** 0.003	0.145** 0.012	0.0002* 0.081
Colony	0.269 0.684	0.563*** 0.001	0.674* 0.087	0.156 0.745
Membership	0.729** 0.032	2.481 0.130	0.416** 0.010	0.5098* 0.065
Extension	0.835** 0.021	0.2528** 0.016	0.445** 0.032	2.267* 0.067
Credit	1.4011 0.105	0.374 0.118	2.452 0.192	0.642 0.122
Cons	-3.798** 0.015	6.689** 0.031	9.550 0.302	-3.29** 0.030
Base category	No adaptation			
Number of observation	164			
LR chi2(52)	2241.05			
Log likelihood	-138.37809			
Prob> chi2	0.0000			
Pseudo R-Square	0.4474			

Notes: *, **, *** = significant at 10%, 5%, and 1% probability level, respectively

TABLE 6
MARGINAL EFFECTS FROM THE MNL CLIMATE ADAPTATION MODEL

Variables	Additional feeding	Forage planting	Skip harvesting	Beehive shade
	Coef. P-value	Coef. P-value	Coef P-value.	Coef. P-value
Sex	1.141 0.102	0. 048 0.107	0 .337 0.481	0.337 0.600
Age	0.113 0.519	0.562*** 0.006	0.891*** 0.000	0.699*** 0.000
Education	0.298** 0.01	0.0032 0.731	1.446 0.171	0.745*** 0.000
Family	0.939 0.303	0.233*** 0.003	0.194 0.757	0.208 0.349
Farm size	0.217 0.306	0.127** 0.02	0.569 0.486	0.776*** 0.000
Livestock	1.343 0.435	0.259 0.845	1.215 0.225	0.123 0.485
Totincome	0.935** 0.023	-0.040*** 0.002	0.657 0.565	0.000 0.720
Perception	0.267*** 0.001	0.217 0.610	0.287** 0.038	0.578*** 0.005
Colony	1.603 0.262	0.286 0.108	0.985 0.161	0.411 0.405
Membership	0.646*** 0.000	0.000 0.72	0.953*** 0.000	0.467*** 0.001
Extension	0.021*** 0.007	0.149** 0.014	0.012 0.916	0.179*** 0.009
Credit	0.999 0.486	0.092 0.817	0.012 0.916	0.259 0.845
Agroecology	0.017 0.835	1.603 0.262	0 .149 0.345	0.007 0.923

Notes: *, **, *** = significant at 10%, 5%, and 1% probability level, respectively

3.3.4 Farm size of the respondent

A one hectare increases in the farm size, the likelihood of the beekeepers use honeybee forages plantation and bee hive shade adaptation strategy increases by 12.7% and 77.6% at 5% and 1% level of significance respectively, holding other variables constant (Table 6). This indicates that the bigger the size of the farm, the greater the proportion of land allocated for bee forages.

3.3.5 Total income

MNL result reveals that as one percent increases in income the likelihood of giving additional feeding as adaptation strategy increased by 93.5 % at 5% of significance level. However, a one percent increases in income of beekeeper reduces the likelihood of use improved bee forage planting adaptation strategy by 4.0% at significance level of 1% (Table 6). This is because beekeepers, perhaps, invest their income on other livelihoods activity than buying either honey bee forage seeds or seedlings in the study area. This is probably purchase of improved bee forages seeds and/or seedling is unfamiliar to the study area. Most beekeepers consider beekeeping as secondary activity.

3.3.6 Perception of climate change effect

Beekeepers who notice effect of climate changes are likely to increase taking up additional feeding, jump honey harvesting and bee hive shade adaptation strategy by 26.7%, 28.7% and 57.8% at a significance level of 1%, 5% and1%, respectively, compared to beekeepers who did not notice effect of climate change, holding other variables constant.

3.3.7 Member of beekeeper group

The result reveals being a membership to the beekeeper group would increase the likelihood of use additional feeding adaptation strategy by 64.6% at 1% level of significant. Moreover, as compared to the beekeepers who have no access to

social network, the likelihood of use skip honey harvesting and bee hive shade adaption strategies to climate change increase by 95.3% and 46.7% at 1% and 1% level of significant, respectively (Table 6). During qualitative assessment, focus group discussants also largely cited social networks as an imperative medium of climate information exchange.

3.3.8 Beekeeping extension

Being getting the extension contact is likely to increase the probability of the beekeeper using additional feeding, improved bee forage planting and bee hive shade as adaptation strategies by 2.1%, 14.9% and 17.9 %, at a significant level of 1%, 5% and 1% respectively, higher than those households' who do not have access extension services (Table 6).

IV. CONCLUSION AND RECOMMENDATION

The study used cross-sectional data collected on 164 sample households in the production year 2018/2019, and applied descriptive and econometric approaches to analyze the data.

The majority of respondents in the study area perceived effects of climate change on honey production and they have taken at least one adaptation measure in response to effects of climate change.

The result from the MNL analysis shows that age, education, family size, farm size, total income, perception, member of beekeeping group, and access to beekeeping extension contact of the household heads have a significant influence on beekeepers' choice of climate change adaptation strategies in the study area.

Being a member of beekeeping group would increase the probability of beekeeper to share beekeeping information related to impact of climate change to adjust their beekeeping to adverse effects of climate change. Hence, concerned bodies either at district level, zonal or higher level should encourage beekeepers to participate in beekeeping organization.

It is important that, extension providers should intensify the provision of beekeeping extension services by insuring increased interaction between smallholder beekeepers and extension agents to complement indigenous knowledge from fellow beekeepers.

Increasing promotion of agroforestry and beekeeper's access to improved bee forages provision along with develop micro-irrigation to enhance their adaptive capacity and long-term resilience to adverse impacts of climate change and variability is very important.

Amidst changing climate and dwindling water availability, the introduction and dissemination of less water consuming bee forage varieties, drought tolerant, shorter cycle, and higher bloom and with good nectar and pollen in the area is important.

$REDD^{++}$ has a potential has to support various adaptation activities in the developing countries. In so doing, country will be able to address underlying drivers of deforestation and forest degradation while supporting communities to adapt.

As a policy issue to support adaptation, the need for the government to enhance collaboration with a spectrum of stakeholders such as civil society and the private sector in ensuring that smallholder beekeepers have access to appropriate information, and training on beekeeping activity related climate change adaptation strategies is very necessary.

ACKNOWLEDGEMENTS

I would like to thank Prof. Fikadu Beyene and Dr. Tesfaye Lemma for their un-reserved effort, insightful and constructive comments for making my paper valuable. I am also highly grateful to the Oromia agricultural research institute for logistic support to conduct this research and I also thank my sample respondents for their valuable cooperation during data collection in responding to all questions with patience and gave the necessary information for this research work.

REFERENCES

[1] Anderson S, Morton J, Toulmin C,"Climate change for agrarian societies in dry lands: Implications and future pathways",Mearns R, Norton A, editors. Social dimensions of climate change: Equity and vulnerability in a warming world. World Bank Publications; p. 199–230, 2010.

[2] Fikreyesus, D., Kaur, N., Kallore, M., Ayalew, L."Public policy responses for a climate resilient green economy in Ethiopia.International Institute on Environment and Development Research Report".London, 2014.

[3] Fernando, C., Otesbergue, A., and Blochtein, B, The dilemma of agricultural pollination in Brazil:Beekeeping growth and insecticide use. PloS one, 13(7),2018

[4] Potts, S. G., Jacobus C. B., Claire Kremen, Peter Neumann, Schweiger,O. and William E.K, Global pollinator Proceedings of the National Academy of Sciences, 104 (50), 2010, pp.19680–19685.

[5] Klein A.M., Vaissiere B.E., Cane J.H., Steffan-Dewenter I., Cunningham S.A., Kremen C. and Tscharntke T, "Importance of pollinators in changing landscapes for world crops," Proceeding of royal Society of London B, biological Science, 274 (1608), 2007,pp.303-313.

[6] Akessa, D.W. Driving socio-economic transformation in Africa. The role of commercial beekeeping. Situation Paper ApiTrade Africa 2016 held in Kigali, Rwanda from 21st to 26th September, 2016.

[7] Barons, M.J., Hanea, A.M., Wright, S.K., Baldock, K.C., Wilfert, L., Chandler, D., Datta, S., Fannon, J., Hartfield, C., Lucas, A. and Ollerton, J, Assessment of the response of pollinator abundance to environmental pressures using structured expert elicitation. Journal of Apicultural Research, 57(5), 2018,pp.593-604.

[8] S. R. Carpenter, R. DeFries, T. Dietz. "Millennium ecosystem assessment: research needs," Science, vol. 314, no. 5797, pp. 257–258, 2006.

[9] McCarthy, N., Lipper, L. and Branca, G, Climate-smart agriculture: Smallholder adoption and implications for climate change adaptation and mitigation. Mitigation of Climate Change in Agriculture Working Paper, 3, pp.1-37,2011.

[10] Melka, A., Scheffran, Jet, Schneider, U.A and Ashfaq, Farmers' perceptions of adaptation strategies to climate change and their determinants: The case of Punjab province, Pakistan. pp.225–243,2015.

[11] Abraham Belay, Recha, J. W., TeshaleWoldeamanuel and Morton, J. F, Smallholder farmers' adaptation to climate change and determinants of their adaptation decisions in the Central Rift Valley of Ethiopia.Agriculture and Food Security, pp.1–13, 2017.

[12] Samuel B, How many subjects does it take to do regression analysis? Multivariate behavioral research, 26(3), 1991, pp.499-510.

[13] Greene H.W, Econometric Analysis, 5th Edition, Pearson Education, Inc., Upper Saddle River, New Jersey, USA, 2003,P. 720-723.

[14] Gujarati D, Basic Econometrics, 4th edition, McGraw Hill Book Company, New York,2003.

[15] Kumsa T and Gorfu B, Beekeeping as integrated watershed conservation and climatic change adaptation: An action research in Boredo Watershed. Journal of Earth Science and Climate Change 5(7), p.1, 2014.

[16] Howland Blackiston, Beekeeping for Dummies, 3rd edition,2017.

[17] Melaku, B., Girmay, M. and Bereket, G, Identifying beekeepers' adaptation strategies in response to climate change in Tigray. Ethiopia Wudpecker Journal of Agricultural Research, 2, p.5.2013.

[18] Deresa, T.T., R.M. Hassan, and C. Ringler, Perception of adaptation to climate change by farmers in the Nile basin of Ethiopia. The Journal of Agricultural Science 149: pp.23-31, 2011.

[19] Gbetibouo, GlwadysAymone, Understanding farmers' perceptions and adaptations to climate change and variability: The case of Limpopo Basin, South Africa. Vol.849.international Food Policy Research Institute, 2009.

Bleaching of Melanomacrophages from Tissues of Ectothermic Vertebrates for Later Use of Immunohistochemical and in Situ Hybridization Technique

Ana Maria Cristina Rebello Pinto da Fonseca Martins[1], Luara Lucena Cassiano[2], Marcia Helena Braga Catroxo[3], Marcio Hipólito[4], Rodolpho R. C.T.E.S.F Costa[5]

[1,3,4,5]Laboratório Interinstitucional de Sanidade em Aquicultura /Instituto Biológico/São Paulo/SP/Br
[2]Laboratório de Microscopia Eletrônica /Instituto Biológico/São Paulo/SP/Br
[1,2,3,4,5]Centro de Pesquisa em Sanidade Animal, Instituto Biológico, Agência Paulista de Tecnologia dos Agronegócios – Secretaria de Agricultura e Abastecimento do Estado de São Paulo, Brasil

Abstract— *Due to the large quantity of melanomacrophages in the organs of the ectothermic vertebrates, with special interest in the ranids and fish, with their brownish melanin granules, we decided to test the MELANIN removal technique, in order to facilitate the observation of the organ fragments in the slides, under the direct light optical microscope, when using the antibodies and biotinylated probes.*

Thus, the melanin bleaching study favored the visualization of the diaminobenzidine chromogen (DAB) without interfering with the antigen-antibody affinity of immunohistochemistry and without interfering with the technique by which specific nucleotide sequences are identified in histological sections. (of DNA or RNA, endogenous, bacterial or viral).

This bleaching of melanin from tissues avoided false positive results, without interfering with the IHQ and ISH techniques for Mycobacterium spp and Francisella spp in fish.

Keywords— Bleaching, ectothermic vertebrates, IHQ and ISH techniques, melanomacrophages.

I. INTRODUCTION

Fish, especially teleosts as well as amphibians, share with the ectothermic vertebrates the existence of an extra-cutaneous pigment system consisting of large, irregular cells in various tissues and organs that can produce and storing melanin within them. This variable amount of melanomacrophages centers or macrophage aggregates are usually found on a larger scale within the endothelial reticulum in the hematopoietic matrix, in the spleen and kidney, but also in the liver (MESSEGUER et al., 1994) and to a lesser extent in the submucosa of the intestine, thymus, gills, brain and gonads of teleosteal fish. The emergence of these structures is related to several factors and conditions, such as organ, age, nutritional status, anatomopathological conditions, as well as environmental changes such as in the processes of detoxification by pollutants and cytoprotective functions related to free radicals.

In the liver, kidneys and spleen, the MMs may contain melanin, cellular fragments, hemosiderin granules and lipofucsine residues, mainly due to their functions of cellular debris sequestration and potentially toxic materials, in addition to participating in the response of fishes and ranids to infectious agents such as fungi and bacterias (STEINEL, BOLNICK, 2017)

The IHQ technique uses the enzyme peroxidase and DAB (3'-3-diaminobenzidine tetrahydrochloride) as the most common and inexpensive chromogen and can have its reaction visualized in a direct light optic microscope, allowing, for example, the diagnosis of various agents' infectious diseases. DAB, however, presents as a marker of deep brown color and may become indistinguishable from the brownish granules of melanin, causing diagnostic errors when viewed under the microscope.

The ISH reaction allows to accurately localizing in the paraffin or frozen tissue, a specific gene or its transcripts. The technique also allows to associate the presence of DNA or mRNA of microorganisms with the morphology, or even to relate the presence of genes and their transcripts with the pathological processes. Thus, there is the association with immunohistochemistry and the ribosondes can be used to study, for example, viral, bacterial, etc. infections, and to differentiate productive viral infections from non-productive ones.

The purpose of this work was to standardize a simple and efficient protocol to bleach the melanin in heavily pigmented tissues. The bleaching technique allowed a correct observation of the chromogen in the antibody-antigen (IHQ) reaction and in the ISH probes in the tissue, not interfering with their affinity and thus avoiding false positives.

II. MATERIAL AND METHODS

Fragments of fish organs collected between apparently healthy animals or diseased animals were fixed in 10% formalin, dehydrated in increasing sequence of alcohols and diaphanized in xylol. After embedded in paraffin, histological sections of 4.5 microns in thickness were glued on silanized slides. With each fragment 3 slides were made. Thus, in 1/3 of the slides the IHC technique was used to search for mycobacterium or francisella using organ fragments the same as the control fragment without adding the antibody. (protocol: blocking the endogenous peroxidase with 3% H_2O_2), the slides were washed with distilled water, followed by a bath with phosphate buffer (0.1 M PBS) at room temperature, 100 µl of the anti-mycobacterium and anti-francisella monoclonal antibodies (Synapse Biotechnology Ltd.) with Dako background reducer (Code S3022) were applied in each slide and incubated in a humid chamber for 18 hours in a refrigerator (2-8oC) .After this period, two washes were carried out with buffer. The system of visualization used was LSAB® + System-HRP (Dako-code K0690) with adaptation of the protocol suggested by the manufacturer. Incubation times of 20 minutes at the substrate-chromogen system was used as the Liquid DAB + Substrat Chromogen System (Dako - code K3468), incubated at room temperature for each of the reactants, intercalated with two washes with phosphate buffer (0.1M PBS) for 1 minute. for 5 minutes at room temperature, followed by washing in running distilled water. Counterstaining was done with hematoxylin).

The sequence of individual primers for *Francisella* spp. (Hsieh et al., 2007) was FLB16S180f: 5'-GCG-GATTAA-AGG-TGG-CCT (Talaat et al., 1997). Trim-C-3 '(forward primer) and FLB16S465r: 5'-CCT-GCA-AGC-TAT-TAA-CTC-ACAGG-3' (reverse primer) for *Mycobacterium* spp. which were specifically amplified: 924-bp fragments based on T-39 (5'G GCGAACGGGTGAGTAACACG-3') andT-13 (5'-TGCACACAGGCCACAAGGGA-3'). The antigen retrieval slides were pre-treated using a water bath at 40 ° C and buffer diluted for recovery (Dako S1699) for 40 min. When the slides are well cooled, the endogenous peroxidase is blocked at room temperature for 20 min and after enzymatic digestion of the tissues with proteinase k , Dako, at room temperature for 5-15 min. Specific biotinylated probes were mixed including the target DNA or RNA on the tissue and covering they with coverslip. Samples and probes were denatured and hybridized overnight (18 h) in the Dako hybridizer (denaturation at 96 ° C and hybridization at 37 ° C). After the stringency bath, TBST (Tris buffered saline / Tween) was washed. The visualization system used: primary streptoavidin in diluent buffer (Dako - kit code K0690) for 30 minutes in a humid chamber, Biotinil Tiramide reagent for 15 min at room temperature and then secondary streptoavidin for 15 min. All applications will be interspersed with two washes with TBST buffer for 5 minutes. The substrate-chromogen system used will be the Liquid DAB + Substrat Chromogen System (Dako - code K3468), incubated for 5 minutes at room temperature, followed by washing in running distilled water. The counterstaining was done with hematoxylin).

The slides used for the bleaching were immersed in 10% hydrogen peroxide (H_2O_2) in 0.2 mol / L Tris-HCl buffer pH 7.4 for 24 hours at room temperature. During this process, the material was kept in the dark. After this procedure, the normal staining protocol for IHC and ISH was followed.

III. RESULTS

Mycobacterium and Francisella positive animals were easily diagnosed using IHC and ISH techniques on slides in which the fragments were bleached with hydrogen peroxide as compared to those that were not cleared. (Figure 1, 2 and 3).

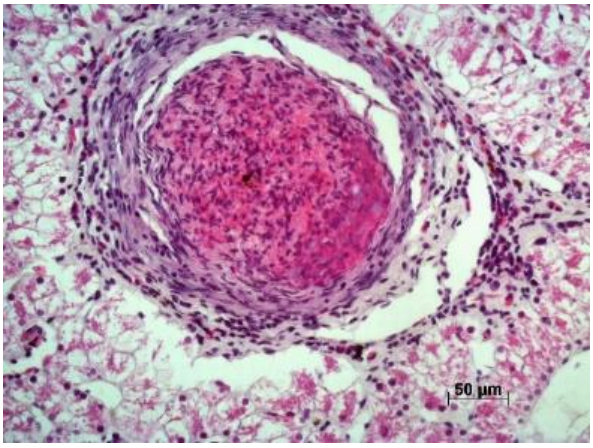


FIGURE 1: No melanin after treatment X200

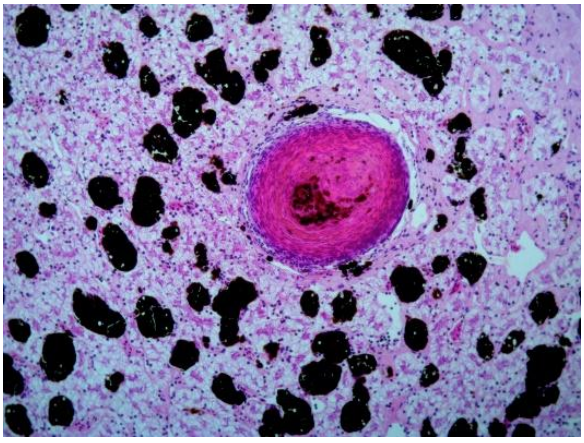


FIGURE 2: With melanin in the granuloma and in melanomacrophages X100

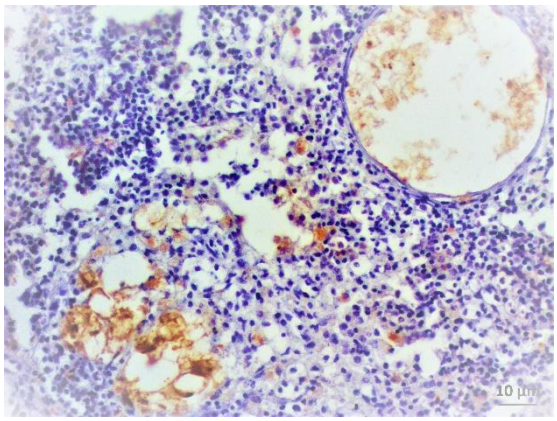


FIGURE 3: Photomicrograph of positive spleen for *Francisella spp*, after treatment, with the in-situ hybridization technique X 400

IV. DISCUSSION AND CONCLUSION

Among the cutaneous pigment macromolecules, we have melanin, eumelanin and pheomelanin (BILLINGHAM & SILVERS 1960, LING 1974). The extra-cutaneous melanin found in melanomacrophages tissues of ectothermic vertebrates is formed by the conversion of tyrosine into alpha-3, 4-dihydroxyphenylalanine and then into dopaquinone and melanin by tyrosinase (WOOLF & SWAFFORD, 1988). There are numerous functions attributed to melanomacrophages such as: antioxidant function, helping to protect the lipids of cell membranes from free radical attack (STEINEL, NC, BOLNICK, 2017), phagocytosis of resistant pathogens such as spores of parasites and bacterias (ROBERTS, 1975); processing of antigens in the immune response (AGIUS, 1985); destruction, detoxification or recycling of endogenous and exogenous materials (FERGUSON, 1976; ELLIS, 1980, HERRAEZ; ZAPATA, 1986); and the response to foreign bodies, including infectious agents (AGIUS, ROBERTS, 2003). Melanomacrophage centers are associated with the presence of acid-resistant intracellular bacteria such as mycobacteria and parasites such as *Myxobolus* spp. (ROBERTS, 2001). There is evidence that melanomacrophages play a relevant role in the control of myxosporidia infections (SUPAMATTAYA et al., 1993).

Bleaching is by gradual oxidation by hydrogen peroxide (KORYTWSKI & SARNA, 1990) and it was evident from our work that the visualization and analysis of IHQ and ISH for francisella and mycobacterium with DAB chromogen use will not be overestimated leading to false positives. This is especially interesting when doing computerized image analysis. These same results were obtained by SILVA, A P and collaborators, in 2011, using the melanin whitening in the epidermis of the South American sea lion for later application of enzymatic immunohistochemistry. It is concluded to be an effective whitening technique for any histological or histopathological study in any animal tissue with melanin.

ACKNOWLEDGEMENTS

Thanks to FAPESP.

REFERENCES

[1] Steinel, N.C.; Bolnick ,D.I. **Melanomacrophage Centers As a Histological indicator of immune Function in Fish and Other Poikilotherms**. Front Immunol.; 8: 827,2017.

[2] Messeguer, J., Lopezruiz, A., Esteban, M., **Melanomacrophages of the seawater teleosts, sea bass (Dicentrarchus labrax) and gilthead seabream (Sparus aurata) – morphology, formation and possible function**. Cell Tiss. Res. 277, 1–10, 1994.

[3] Hsieh CY,Wu ZB, Tung MC, Tsai SS**. PCR and in situ hybridization for the detection and localization of a new pathogen Francisella-like bacterium (FLB) in ornamental cichlids.** Dis Aquat. Organ. 2007; 75:29–36, 2007.

[4] Talaat AM, Reimschuessel R, Trucksis M**. Identification of mycobacteria infecting fish to the species level using polymerase chain reaction and restriction enzyme analysis**. Vet. Micro., 58: 229-237,1997.

[5] Billingham R.E.; Silvers W.K. **The melanocytes of mammals**. Quart. Rev. Biology 35(1):1-40, 1960

[6] Ling J.K. **The integument of marine mammals**, p.1-42. In: Harrison R.J. (Ed.), Functional Anatomy of Marine Mammals. Vol.2. Academic Press, London.,1974

[7] Woolf C.M. & Swafford J.R. 1988. **Evidence for eumelanina and pheomelanin producing genotypes in the arabian horses.** J. Heredity V 79, Issue 2, 100–106,1988

[8] Roberts RJ. **Melanin-containing cells of teleost fish and their relation to disease**. In: Ribelin WE, Migaki G, editors. The Pathology of Fishes. University of Wisconsin p. 399–428, 1975.

[9] Agius C.**The melano-macrophage centres in fish: a review.** In: Fish Immunology (ed. by M.J. Manning & M.F. Tatner), pp. 85–105. Academic Press, London, 1985

[10] Ferguson HW. **The relationship between ellipsoids and melano-macrophage centres in the spleen of turbot (Scophthalmus maximus).** J Comp Pathol 86:377–80. 1976

[11] Ellis AE. **Antigen-trapping in the spleen and kidney of the plaice Pleuronectes platessa** L. J Fish Dis 3:413–26, 1980

[12] Herráez MP, Zapata AG. **Structure and function of the melano-macrophage centres of the goldfish Carassius auratus**. Vet Immunol Immunopathol 12:117–26. doi:10.1016/0165-2427(86)90116-9,1986.

[13] Roberts R.J. **Fish Pathology**, 3rd edn. W.B. Saunders, Philadelphia, PA.,2001

[14] Supamattaya, K.; Fischer-Scherl, T.; Hoffmann, R.W.; BoonyaraTpalin, S**. Light and electron microscopic observations on presporogenic and sporogenic stages of Sphaerospora epinepheli (Myxosporea) in grouper (Epinephellus malabaricus**). Journal of Eukaryotic Microbiology, v. 40, n. 1, p. 71-80, 1993

[15] Silva,A.P.; Silva ,G.R; Cogliati,B;Dias,A.S.M.; Le Bas,A.E. ,Hernandes-Blazquez, F.J. **Bleaching of melnin in the epidermis of South American fur seal and its application on enzyme immunohistochemistry**. Pesq. Vet.Bras. v 31, n 3, 267–270 2011.

[16] Agius C, Roberts RJ. **Melano-macrophage centres and their role in fish pathology**. J Fish Dis 26:499–509, 2003.

[17] Korytowski W. & Sarna T.. **Bleaching melanin pigments: A role of copper ions and hydrogen peroxide in autooxidation and photo-oxidation of synthetic dopa-melanin**. J. Biol. Chem. 265(21):12410-12416, 1990.

Management of Brown Spot Disease of Rice and Studies of Growth Rate of Disease on Application of Different Synthetic Fungicides by using Different Statistical Tools

David Kamei[1], Archana U. Singh[2], Adam Kamei[3]

[1]KVK, ICAR, Senapati District, Manipur
[2]ICAR, IARI, New Delhi
[3]NCIPM&FW, Ministry of Agri, GOI, Manipur Centre, Imphal

Abstract— *The in-vivo test of selected fungicides against brown spot disease of rice and studies on growth rate of disease incidence by using different statistical tools was carried out during the crop seasons, kharif (2014-15) and (2015-16). The pool mean results data of two crop seasons revealed that among the synthetic fungicides evaluated against per cent disease incidence, minimum disease index (PDI) was found in Propiconazole (7.39) with maximum disease reduction of 72.75% over the untreated control followed by Propineb (7.91) and Myclobutanil (8.84) with per cent disease reduction of 70.83 and 67.40 respectively over the control. Among the fungicides treatment maximum disease incidence was observed in Thiophanate (16) followed by Carbendazim (10.96) with per cent disease reduction of 41 and 59.58 over untreated control. The studies on rate of growth of disease severity by using linear and non linear parameters among the synthetic fungicides found that lowest average growth rate during the first crop seasons (2014-15) was observed in Propiconazole (0.124) at 10 days intervals of disease progression analysis studies. Similarly in the following crop season (2015-16) also lowest average growth rate of untransformed and transformed model was observed in Propiconazole (0.069). The analysis thus obviously confirmed that among the different synthetic fungicides tested, Propiconazole was the most effective and most promising fungicides in managing the brown spot disease incidence of rice*.

***Keywords— Brown spot disease, rice, synthetic fungicides, minimum disease index*.**

I. INTRODUCTION

Rice is a staple food to more than half of the world population around 4 billion people. It is a staple food to two third of Indian (Rout and Tiwari, 2012). It is estimated that 3.4 billion people eat rice everyday (*irri.org/news-and-event/news/scaling-sustainable-rice-farming-practices-achieve-food-security-asia, 2020*).

In terms of global rice production India remained as single second largest country with 118.00 million metric tons and China being world number one with 146.73 million metric tons. (*worldagriculturalproduction.com/crop/rice, aspx, Apr.16, 2020*). Although India held a prominent position in global rice areas and production, the productivity per unit area by world standard is still low with average productivity of about 2.39 t/ha, whereas, in case of China it is 6.71 t/ha. One major factor for low productivity of rice in India is due to pest and disease incidence. Several pathogenic and non pathogenic diseases caused an extensive economic loss to rice crops. The losses due to rice diseases have been estimated to be 10-15% in general (Kandhari, 2005). Among the pathogens, fungi alone account for nearly 30 diseases of rice in the country (Rangaswami *et al.* 2002). The brown spot disease of rice incited by *Helminthosporium oryzae* is one major fungal diseases that caused yield loss of upto 45% when no coverage of plant protection were given.

(*http://www.knowledgebank.irri.org/training/factsheets/pestmanagement/diseases/item/brown-spot*). Brown spot disease of rice has been reported to occur in all rice growing countries including Japan, China, Burma, Sri Lanka, Bangladesh, Iran, Africa, South America, Russia, North America, Philipines, Saudi Arabia, Australia, Malaysia and Thailand, (Ou, 1985; Khalili, *et al*. 2012). In India it was known to occur in all rice growing states but was found more severe in dry and direct seeded rice in the state of Bihar, Chhatisgarh, Madhya Pradesh, Orissa, Assam, Jharkhand and West Bengal (Gangopadhyay, 1983; Sunder, *et al*., 2014).

At present era of agriculture, predominant means of crop protection is the use of chemicals. However, the efficacy of existing pesticides available in the open market always need to be thoroughly evaluated so as to deal effectively with the target pest without loss of time, energy and capital, since most chemicals are costly and its indiscriminate use has also resulted a serious ecological and adverse effect on the human and animal health which has become a major global issue. A judicious

application of pesticides needs to be advocate at the highest level through researched an extension activity for monitoring economic losses as well as copping with the environmental issue. Hence, the present work was undertaken to resolve issue of menace of brown spot disease of rice and indiscriminate use of chemical through proper evaluation of selected chemicals by using statistical tools such as, Logistic growth model and Gompertz model.

Disease progress curves over time have been referred to be as the "*signature*" of the epidemic and represent an integration of an all host, pathogen and environmental effects occurring during the epidemic (Campbell and Madden, 1990). A disease progress curve shows the epidemic dynamics over time (Agrios, 2005). This mathematical tool can be used to obtain information about the appearance and amount of inoculums, changes in host susceptibility during growing period, weather events and the effectiveness of cultural and control measures. Growth models provide a range of curves that are often similar to disease progress curves (Van Maanen and Xu, 2003) and represent one of the most common mathematical tools to describe temporal disease epidemics (Xu, 2006). The growth models commonly used are: Monomolecular, Exponential, Logistic and Gompertz (Zadok and Schein, 1979; Nutter, 1997; Nutter and Parker, 1997; Xu, 2006). A brief description of each growth model is presented as follows: Equations with linear parameters from each of four models of Richard's family of growth curves, i.e. monomolecular $(ln[1(1-y)]=ln[1/(1-y_0)]+r_Mt$, $logistic(ln[/(1-y)]= ln[y_0/(1-y_0)]+r_Lt$,$log\text{-}logistic(ln[y/($log-logistic $logistic(ln[/(1-y)]= ln[y_0/(1-y_0)]+r_Lt$ and Gompertz (-ln[-ln(y)] = -ln[-ln(yo)]+ rGt were employed as predicted equations to statistically compare linearly transformed data (Campbell and Madden, 1990; Nutter and Parker, 1997). Variables were: y=Mean severity of disease(S) as a proportion from 0 to 1 at time t, yo=the initial disease level and r*=rate of disease increase for each model. After the regression analysis, goodness of fit of the models was determined by examining the coefficient of determination $R^{2,}$ which is the proportion of the variation in the data accounted for by the variation in the data error of estimates (SEE), and the plot of the standardized residuals versus the predicted values. An $R^2 \geq$ 80% is the desirable: if $R^2 \leq 50\%$, the model fits the data poorly.

To compare models using different transformation of the dependent variables for goodness-of-fit, predicted transformed y was back-transformed and the co-efficient of determination calculated based on these values (R^{*2}) (Campbell and Madden, 1990). Having selected the most suitable models, regression analysis was performed between observed and back-transformed dependent variables. Analysis of variance (ANOVA) was used to reveal any significant difference between the regions in rated parameters.

II. METHODOLOGY

2.1 *In-vivo* test

Field trial was carried out in the experimental plot of Department of Plant Pathology, Allahabad School of Agriculture, SHUATS, Allahabad, U.P., in a consecutive two cropping seasons of kharif (2014-15) and (2015-16) by using a susceptible Manipur paddy cultivar *viz.*, Daram-phou. Field layout were made in Randomized Block Design (RBD) with plot size (2x3) sq. m., a 25 days old seedlings were transplanted with spacing 20 cm (row x row) and 15 cm (plant x plant), with 2-3 seedlings/hill. Five fungicides *viz.*, Thiophanate, Carbendazim, Myclobutanil, Propineb, Propiconazole at 1000ppm were sprayed at 10 days intervals from 48, 58 and 68 days after transplantation of the paddy and when prominent disease symptoms start appearing. Periodical monitoring on fixed plot were performed for obtaining real time data for rice brown spot disease incidence and severity in experimental plots. Observation was made one day ahead of each time of the treatment application and final observation was taken at 10 days after the final or third spray. For measuring disease progress 5 plants per plot were tagged inside of the field borders and one in the centre and top three leaves were taken into consideration in each time of disease rating during observation and data were systematically recorded and maintained as per the standard procedure.

The rating of the disease severity was done by using disease scoring scale of 0-4, based on percentage number of leaves showing symptoms according to Kalloo and Banerjee (2000) [where, 0=No symptoms observed, 1=1-25 % leaf area affected, 2=26-50 % leaf area affected, 3=51-75% leaf area affected and 4=75% and above leaf area affected]. Disease rating was recorded and the percent disease severity was worked out subsequently at every 10 days interval of the growth stage of the crop by following formula (Mc Kinny, 1923):

$$\text{PDI (\%)} = \frac{\text{Summation of numerical ratings}}{\text{Total number of leaves observed} \times \text{Maximum rating grade}} \times 100$$

III. STUDIES ON GROWTH RATE OF DISEASE ON APPLICATION OF DIFFERENT SYNTHETIC FUNGICIDES

3.1 Logistic growth model:

It was proposed firstly by Veshulst in 1838 to represent human population growth. A second type of logistic model was proposed by Van der Plank (1963), being more appropriate for most polycyclic diseases, meaning that there is a secondary spread within a growing season (Forrest, 2007). This growth model is the most widely used for describing epidemics of plant disease (Segarra *et al.*, 2001; Jeger, 2004).

Logistic compound interest: (rate of function)= Ry(l-y),

According to Vander Plank's (1963) equation: dx/dt = QR,

Where, X = the proportion of tissue disease,

R = apparent infection rate,

(l-x) = the proportion of tissue available for infection.

If the total amount of "X" of capital interest varies with time 't', then dt means a very small interval of time, and dx is the very small bit that X increase in that interval. a, k, c, b and 0.05 = constant.

3.2 Gompertz model

This growth model is appropriate for polycyclic diseases as an alternative to logistic models. Gompertz model has an absolute rate curve that reaches a maximum more quickly and declines more gradually than the logistic models (Forrest, 2007) shows examples of disease progress curves represented by growth models, where it can be seen that Gompertz and logistic models have a characteristic sigmoid form and an inflection point meaning secondary inoculation or plant-to-plant spread within the crop in contrast to monomolecular model, which does not have inflection point. The exponential model presents a very small value at the beginning comparing with the other models and latter it increases exponentially. In general, growth models that incorporate few variables to describe temporal disease dynamics have a good performance; however, this kind of models sometimes do not satisfy the acquiring process of key characteristics because they frequently ignore relevant variables that affect the epidemic development (Xu, 2006), *e.g.* host growth, fluctuating environmental condition, length of latent and infectious period, etc. Nevertheless, advances in statistical and computing technologies have allowed incorporating several of these kinds of characteristics in order to obtain a more reliable model. It is important to mention that the researchers should be aware of some violations presented in these models by checking if some assumptions about the epidemic are not met and if there are some inevitable violations; they must try to find means to reduce such violations in order to diminish the bias and to correctly interpret results (Xu, 2006). Van der Plank (1960) used exponential, monomolecular and logistic models to describe the development of epidemics. Xu (1999) used a logistic model to forecast and model the apple powdery mildew provoked by *Podosphaera leucotricha*. The work presented by Mersha and Hau (2008) uses logistic and Gompertz models to study the effects of rust bean on host dynamics of common bean in controlled greenhouse experiments with and without fungicide sprays. A deep description of these growth models can be found in the book written by Campbell and Madden (1990).

Gompertz model: $X(t)=c \exp(-b \exp(-at))+e$, Where X(t), the disease severity at time t; a, b, c, d the parameters, and e, the error term.

Software packages used (for growth model): Curve expert professional

IV. RESULTS AND DISCUSSION

The results obtained during the course of investigation are presented in the following tables and figures and inferences were made there on:

4.1 Efficacy of selected fungicides on per cent disease incidences of brown spot of rice

TABLE 1
SELECTED FUNGICIDES AND PER CENT DISEASE INCIDENCE OF BROWN SPOT OF RICE DURING FIRST CROPPING SEASON (2014-15)

S. No.	Treatment	PDI crop season (2014-15)					
		BS* a	AFS* b	ASS* c	ATS* d	Mean (abcd)	% Control
1.	T_0 (Control)	8.2	22.43	29.15	32.76	**28.11**	-
2.	T_1 (Thiophanate)	7.8	12.01	18.17	20.91	**17.03**	**39.41**
3.	T_2 (Myclobutanil)	9.16	6.96	11.83	11.69	**10.16**	**63.52**
4.	T_3 (Carbendazim)	8.6	6.91	15.01	13.33	**11.75**	**58.19**
5.	T_5 (Propineb)	8.2	4.69	10.72	10.39	**8.6**	**69.40**
6.	T_6 (Propiconazole)	7.8	4.44	9.92	8.94	**7.76**	**72.39**
	Mean (abcd)	**8.29**	**9.57**	**15.80**	**16.33**	**12.50****	-
	S.Ed (±)	**1.7**	**0.43**	**0.21**	**0.22**	**0.71**	**1.9**
	CD (0.05%)	**2.03 (NS)**	**0.61**	**0.29**	**0.32**	**0.40**	**5.60**

Mean value of four replication
BS-before spray, AFS-after first spray, ASS-after second spray, ATS-after third spray,
bcd-mean PDI value three observation after the spray
abcd-mean PDI value of four observation**
NS-non significant

The data presented on Table 2, is the per cent disease incidence of brown spot disease of rice and the selected fungicides at three consecutive schedule of spray at 48, 58 and 68 days and subsequent observation taken at 10 days interval *i.e*. 47, 57, 67 and 77 days after transplanting of the first cropping season (2014-15).

The results data revealed that before the treatment was applied there was no significance different among the treatment and between non treatment control plots concerning disease incidences. However, observation taken at 9 days after the first treatment found per cent disease incidence was lowest in Propiconazole (4.44) followed by Propineb (4.69), Myclobutanil (6.96) and highest incidence was observed in Thiophanate and Carbendzim treatment with per cent disease incidence of (12.01) and (11.01) respectively over the untreated control (22.43). However, all treatment fungicides were found significantly different among themselves and the untreated control. Similarly in the following second and third treatment on each time of observation taken at 9 days after the treatment application it was observed that per cent disease incidence (PDI) was always found lowest in treatment with Propiconazole followed by Propineb and Myclobutanil and maximum disease incidence was observed in Thiophanate and Carbendazim. It is also evident from the mean PDI value of treatment (bcd) that lowest per cent disease index was found in Propiconazole (7.76) with per cent disease control (72.39) followed by Propineb (8.6) and Myclobutanil (10.16) with per cent control (69.40) and (63.52) respectively over control, whereas, maximum per cent disease index was found in Thiophanate (17.03) and Carbendazim (11.75) with per cent disease control of (39.41) and (58.19) respectively over the untreated control. However, in all cases all treatment fungicides were found significantly different among themselves and the untreated control.

TABLE 2
SELECTED FUNGICIDES AND PER CENT DISEASE INCIDENCE OF BROWN SPOT OF RICE DURING SECOND CROPPING SEASON (2015-16)

S. No.	Treatment	PDI crop season (2015-16)					
		BS* a	AFS* b	ASS* c	ATS* d	Mean (bcd)	% control
1.	T_0 Control	9.16	20.68	26.42	31.29	**26.13**	-
2.	T_1 Thiophanate	8.32	9.21	16.36	19.37	**14.98**	**42.67**
3.	T_2 Myclobutanil	8.36	5.94	7.59	9.04	**7.52**	**71.22**
4.	T_3 Carbendazim	7.64	6.91	12.55	11.1	**10.18**	**61.04**
5.	T_4 Propineb	7.65	4.70	8.31	8.70	**7.23**	**73.09**
6.	T_5 Propiconazole	8.12	4.23	8.76	8.12	**7.03**	**73.09**
	Mean (abcd)	**8.20**	**8.61**	**13.33**	**14.60**	**11.18****	-
	S.Ed (±)	**1.32**	**0.17**	**0.22**	**0.19**	**0.19**	**0.75**
	CD (0.05%)	**3.04 (NS)**	**0.51**	**0.68**	**0.57**	**0.58**	**2.32**

Mean value of four replication
BS-before spray, AFS-after first spray, ASS-after second spray, ATS- after third spray,
bcd- Mean PDI value three observation after spray
abcd- mean PDI value of four observation**
NS - Non significance

The data presented on Table 2, are selected fungicides on brown spot disease incidence observation taken at 10 days interval *i.e.* 47, 57, 67 and 77 days after transplanting of the second cropping season (2015-16).

The results data revealed that before the treatment was applied there was no significance among the treatment and untreated control plots concerning per cent disease incidence. However, observation taken at 9 days after the first sprayed it was observed that per cent disease incidence was lowest in Propiconazole (4.23) followed by Propineb (4.70), Myclobutanil (5.94) and highest incidence was observed in Thiophanate and Carbendzim treatment with per cent disease incidence of (9.21) and (6.91) respectively over the untreated control (20.68). However, all treatment fungicides were found significantly different among themselves and the untreated control. Similarly in the following second and third treatment and on each time of observation taken at 9 days after the treatment application it was observed that per cent disease incidence (PDI) was always found lowest in treatment with Propiconazole followed by Propineb and Myclobutanil and maximum disease incidence was observed in Thiophanate and Carbendazim. It is also evident from the mean PDI value of treatment (bcd) that lowest per cent disease index was found in Propiconazole (7.03) with per cent disease control (73.09) followed by Propineb (7.23) and Myclobutanil (7.52) with per cent control (73.09) and (71.22) respectively over control, whereas, maximum mean per cent disease index value bcd was found in Thiophanate (14.98) and Carbendazim (10.18) with per cent disease control of (42.67) and (61.04) respectively over the untreated control. However, in all cases all treatment fungicides were found significantly different among themselves and with the untreated control.

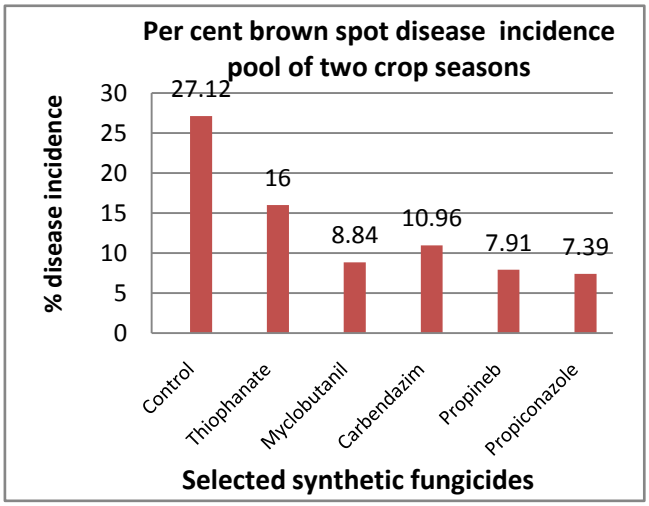


FIGURE 1: Fungicides and per cent disease incidence pool data of two crop seasons

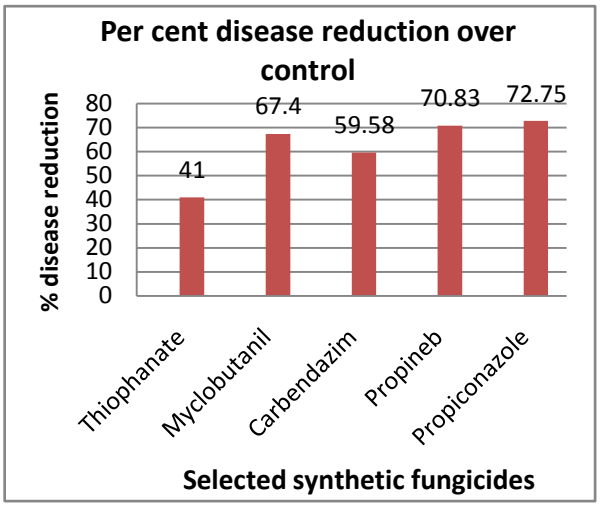


FIGURE 2: Fungicides and per cent disease reduction over control

TABLE 3

SELECTED FUNGICIDES AGAINST PER CENT DISEASE INCIDENCE OF BROWN SPOT OF RICE (POOL DATA OF TWO CROPPING SEASONS)

S. No.	Treatment	PDI		Pooled mean	% disease reduction
		2014- 15	2015-16		
1.	T_0 Control	28.11	26.13	27.12	-
2.	T_1 Thiophanate	17.03	14.98	16.00	41.00
3.	T_2 Myclobutanil	10.16	7.52	8.84	67.40
4.	T_3 Carbendazim	11.75	10.18	10.96	59.58
5.	T_4 Propineb	8.6	7.23	7.91	70.83
6.	T_5 Propiconazole	7.76	7.03	7.39	72.75
	S.Ed (±)	**0.28**	**0.19**	**0.23**	**0.21**
	CD	**0.40**	**0.58**	**0.49**	**0.53**

The data presented in the above Table 3, and fig. 1&2, is the results data, pooled of two consecutive cropping seasons, kharif (2014-15) and (2015-16) of the selected fungicides treatment against per cent disease incidence and the per cent disease reduction index over untreated control. Among the treatments minimum brown spot incidence was recorded in Propiconazole (7.39) with per cent disease reduction (72.75), followed by Propineb (7.91), Myclobutanil (8.84) with per cent disease reduction (70.83) and (67.40) respectively over the control. Among the treatment fungicides least significant disease incidence was recorded in Thiophanate (16) with per cent disease reduction of (41%) followed by Carbendazim (10.96) with per cent disease reduction (59.58%) over the untreated control with percent disease incidence of (27.12).

The analysis of the above results data of the *in-vivo* test during the crop seasons (2014-15) and (2015-16) revealed that all selected fungicides significantly inhibit the disease incidence in all the three schedule of spray. However, among the treatments highest significant per cent reduction of brown spot disease incidence was recorded in Propiconazole followed by Propineb, Myclobutanil and minimum significant reduction was found in Thiophanate followed by Carbendazim. However, all fungicides were found significantly different in reducing the per cent disease incidence over the untreated control. Our present finding are in corroborate with that of Percich (1989) who reported that foliar application with Propiconazole was found to have better results in management of brown spot disease of rice. Pannu *et al*. (2003) also reported that application of Propiconazole was found most effective against brown spot disease. Moletti *et al*. (1996) reported that application of Iprodione and Propiconazole was most effective against brown spot disease, whereas Celmer *et al*. (2007) reported that

Trifloxystrobin + Propiconazole can effectively control the brown spot diseases of rice. Kumar and Rai (2008) also reported that application of Antracol or Propineb and RIL-FA 200SC can effectively reduced the brown spot incidence of rice. Sunder *et al*. (2010) reported that spraying of Hexaconazole and Propiconazole at early booting stage considerably reduced both leaf spot and stalk rot phase of brown spot disease of rice. The results data also found disease severity was more during the first cropping season 2014-15 as revealed by higher mean PDI value of four observation abcd (12.50**) whereas, in the second cropping seasons (2015-16) with lower mean PDI value of four observation abcd (11.18**).

4.2 Analysis on growth rate of brown spot disease incidences of rice in relation to application of different synthetic fungicides

TABLE 4
STUDIES OF DISEASE GROWTH RATE USING DIFFERENT LINEAR AND NON-LINEAR PARAMETERS DURING (2014-15)

Disease Growth Rate (2015-16)						
Fungicides	**DS**	**% Disease**	**Arcsine**	**Logit**	**Gompit**	**Average**
Thiophanate	0.684	0.006	0.567	0.052	0.061	0.274
Myclobutanil	0.327	0.003	0.006	0.05	0.018	0.081
Carbendazim	0.282	0.002	0.319	0.015	0.041	0.132
Propineb	0.233	0.002	0.028	0.043	0.043	0.070
Propiconazole	0.260	0.002	0.029	0.041	0.014	0.069
Control	0.960	0.009	0.073	0.794	0.799	0.527

TABLE 5
STUDIES OF DISEASE GROWTH RATE BY USING DIFFERENT LINEAR AND NON-LINEAR PARAMETERS DURING (2014-15)

Disease Growth Rate (2014-15)						
Fungicides	**DS**	**% Disease**	**Arcsine**	**Logit**	**Gompit**	**Average**
Thiophanate	0.596	0.006	0.561	0.067	0.026	0.251
Myclobutanil	0.327	0.003	0.372	0.054	0.018	0.155
Carbendazim	0.799	0.002	0.293	0.044	0.014	0.230
Propineb	0.282	0.002	0.319	0.015	0.015	0.127
Propiconazole	0.260	0.002	0.303	0.043	0.014	0.124
Control	0.960	0.009	0.805	0.089	0.037	0.380

V. CONCLUSION

In this present investigation, disease severity data were transformed to determine the disease progression. Disease data were subjected to untransformed disease data and transformed to obtained disease percent, and arcsine, logit and gompit transformation. The growth rate was calculated based on untransformed and transformed of the disease data in order to detect the changes taking place for formulating effective management strategies. It is indicated that Propiconazole (0.124) showed the most effective fungicide among treated chemicals, as indicated by the lowest growth rate was observed among the treated chemicals at ten days intervals of disease progression. Here, it was observed that gompit transformation showed lowest among transformed, and it was confirmed that the disease severity growth rate of the target pathogens can be predicted. Considering all the models, the most effective fungicide in minimizing the spread of disease and its severity were observed in Propiconazole treatment. Similarly, in the following year (2015-16) the most promising fungicide showing lowest growth rate was propiconazole as was in the previous year (2014-15), obviously as indicated by the average growth rate values from untransformed and transformed models being observed lowest in propiconazole treatment (0.069) as depicted in Table-3 and Table-4 respectively. Disease progress curves exploiting growth model, described the disease progress in a good way with few weathers factors (Xu, 2006). The disease data subjected on transformed to investigate disease progression was also reported by Gompertz (Kranz, 1974; Berger, 1981) and the logistic transformation (Vander plank, 1963). Plant disease progress was described for Comparison of the Gompertz and logistic equations (Berger, 1981). Similarly, Logistic and

Gompertz models with and without fungicide sprays was also reported to study the effects of rust of bean on host dynamics of common bean in controlled Greenhouse experiment (Hau, 2008). Similar observations have been reported earlier for other patho systems such as wheat leaf rust (Hau and Kranz, (1977), apple scab (Analytis, 1979) and groundnut rust (Das and Raj, 2000).

REFERENCES

[1] Agrios, G. N. (2005). Plant Pathology. Fifth Edition, Elsevier Academic Press, London,UK.

[2] Analytis, S., (1979). Die transformation Van Befallswerten in der quantitative phytopathology. IF Das Liverisie ren von Befallskurven. *Phytopath Z*. **96**: 156-171.

[3] Berger, R. D., (1981). Comparison of the Gompertz and logistic equations to describe plant disease progress. *Phytopathology,* ***71,*** 716–719. doi:10.1094/Phyto-71-716

[4] Campbell, C. L. and Madden, L. V. (1990). Introduction to plant disease epidemiology. John Wiley & Sons, New York, pp 532.

[5] Celmer, A., Madalosso, M.G., Debortoli, M.P.,Navarini,L. and Balardin,R.S., (2007). Chemical control of irrigated rice diseases. *Pesquisa Agropecuaria Brasilleira*. 42: 901-9046.

[6] Chakrabarty, S., Tiedemann, A.V. and Teng, P. S., (2000). Climate change: potential impact on plant diseases. *Environmental Pollution*, 108**:** 317-326.

[7] Chattopadhyay, C., Meena, P.D., Godika, S., Yadav, M.S., Meena, R.L. and Bhunia, C.K. (2005). Garlic bulb extracts a better choice, than chemical fungicides in managing oilseed crop diseases. *J. Mycol. Pl. Pathol*. 35: 574-575.

[8] Dasgupta, M.K. and Chattopadhyay, S.B., (1977). Effect of different doses of N and P on the susceptibility of rice to brown spot caused by *Helminthosporium oryzae*. *Z. Pftanzenkrankh. Pftlanzensch*. 84**:** 276-285

[9] Das, S. and Raj, S.K. (2000). Comparison between Logistic and Gompertz equation for predicting groundnut rust epidemics. *Indian Phytopath*. **53**: 71-75.

[10] Forrest, B.M., (2007). Managing risks from invasive marine species: is post-border management feasible? PhD Thesis, Victoria University of Wellington, Wellington, New Zealand

[11] Gangopadhyay, S., (1982). Current concepts on Fungal Diseases of Rice. Today and Tomorrow's Printers & Publishers, New Delhi, 349pp.

[12] Ghose, R.L.M., Ghatge, M.B. and Subramanian, V., (1960). Rice in India (revised edn.), New Delhi, ICAR, 474pp.

[13] Hau, B. and Kranz, J., (1977). Ein vergleich Vershiedener transformation en von Befallskurven. *Phytopath Z.* **88:** 53- 68.

[14] Khalili, E., Sadravi, M., Naeimi, S. and Khosravi,V.,(2012). Biological control of rice brown spot with native isolates of three trichoderma species. *Braz. J. Microbiol*. 43**:** 297-305

[15] Kranz, J., (1974). The role and scope of mathematical analyse and Modelling in epidemiology. In Epidemics and Plant Diseases, Mathematical analysis and Modelling (Ed. J. Kranz), pp. 7-54 Sringer, New York. 170pp.

[16] Kranz, J. and Royle, D.J., (1978). Perspectives in mathematical modelling of plant disease epidemics,in plant disease epidemiology . Scott PR and Bainbridge A(eds). Blackwell Scientific Publications. Oxford, London, Edingburgh, Melbourne. pp111-120

[17] Kumar, S. and Rai, B., (2008). Evaluation of new fungicides and biopesticides against brown spot of rice. *Indian Agriculturist* 52:117-119

[18] Mc kinney, H.H., (1923). A new system of grading plant diseases. *J. Agric. Res.*, 26: 195-218

[19] Mersha, Z. and Hau, B., (2008). Effect of bean rust (*Uromyces appendiculatus*) epidemics of host dynamics of common beans (*Phaseolus bulgaris*). *Plant Pathol.*, **57:** 674-686.

[20] Molletti, M., Giudici, M.L. and Villa, B., (1996). Rice Akiochi brown spot disease in Italy: agronomic and chemical control. *Informators Fitopathologico*. 46**:** 41-46

[21] Nutter FW, and Parker, SK., (1997). Fitting disease progressive curved EPIMODEL. In exercise in plant disease epemiology . fraci. IJ and Neher DA (eds). APS press, St. Paul, MN, USA, pp.24-28

[22] Ou, S.H. (1985). Rice diseases 2nd edn. CMI, Kew, England, 370pp

[23] Pannu,P.P.S., Mandeep Kaur and Chahal, S.S. (2003).Evaluation of different fungicides against *Helminthosporium oryzae* in vitro and in-vivo conditions. *J.Mycol.Pl.Pathol*. 33: 473 (abstr.)

[24] Percich, J.A., (1989). Comparison of Propiconazole rates for control of fungal brown spot of wild rice by Bipolaris oryzae in the growth chamber. *Plant Dis.* 73**:** 588-589

[25] Pico, A.M. and Rodofil , M., (2002). Pyricularia grisea and Bipolaris oryzae: a preminary study on the occurrence or airborne spores in a field. Aerobiology, 18(2):163-167.[doi: 10.1023/A:102654319130]

[26] Rangawami, G. and Mahadevan, A, (2002): Diseases of crop plants in India, fourth edition IEEE, pp160.

[27] Segarra, J., Jerger, M.J., Van den Bosch, F., (2001). Epidemic dynamics and patterns of plant diseases. *Phytopathology*, 91:1001-1010.

[28] Sangeetha, C.G. and Siddaramaiah, A.L., (2007). Epidemiological studies of white rust downy mildew and Alternaria blight of Indian mustard *(Brassicae juncea* (Linn). (Zern. and loss).*AfricanJ.Agril.Res*.2**:** 305-308.

[29] Sasaki, T. and Burr, B. (2000). International rice genome sequence project: The effort to complete the sequence of rice genome. Current opinion in plant Biology; 3:2, pp138-141

[30] Scherm, H. and Yang, X. B., (1995). Interannual variations in wheat rust development in China and the United States in relation to the El Nino/Southern Oscillation. *Phytopathology*, 85: 970-976

[31] Schoeny,A. Jumel,S. Rouault, F., May, Le C. and Tivoli, B., (2007). Assessment of airborne primary inoculums availability and modelling of disease onset of Ascochyta blight in field peas. *Eur. J. Plant Pathol*. 119**:** 87-97.

[32] Singh Chhidda, Singh Prem., and Singh Rajbir, (2008). Modern Techniques of Raising Field Crops, Second Edn. Pp.68-73

[33] Singh, R. K., Singh, C. V., & Shukla, V. D., (2005). Phosphorus nutrition reduces brown spot incidence in rainfed upland rice. *International Rice Research Notes*, 30(2): 31–32.

[34] Sunder, S., Singh, R. and Dodan,D.S., (2010). Evaluation of fungicides, botanicals and non conventional chemicals against brown spot of rice. *Indian Phytopath.* **63:**192-194

[35] Sunder. S, Ram Singh and Rashmi Agarwal, (2014). Brown spot of rice: an over view. *Indian Phytopath.* 67(3): 201-215

[36] Sutherst, R.W., (1993). Role of modelling in sustainable pest management. In: Pest control and sustainable agriculture. Corey S, Dall D and Mine W (eds.) CSIRO, Australia, pp.66-71

[37] Taylor MC, Hardwick NV, Bradshaw NJ, Hall AM. Relative performance of five forecasting schemes for potato late blight (*Phytophthora infestans*) I. Accuracy of infection warnings and reduction of unnecessary, theoretical, fungicide applications. *Crop Protection*. 2003;22:275283.doi:10.1016/S0261-2194 (02) 00148-5[Cross Ref.]

[38] Van der plank, J.E., (1963). Plant disease epidemics and control. Academic Press. New York. 349pp.

[39] Van der plank, J.E., (1960). Analysis of epidemics. In: Plant pathology. Horsefall J G and Cowling EB (eds). Academic Press, New York, USA.pp.230-287

[40] Van Maanen, A., and Xu, X.M., (2003). Modelling plant disease epidemics, European J.Plant Pathol. **109:** 669-682.

[41] Xu, (2006). Modelling and interpreting disease progress in time. In: The epidemiology of plant disease. Cooke BM, Gareth Jones D and Kaye B (eds) Springer, Dordrecht, The Netherlands, pp. 215-238.

[42] Zadoks,J.C., and Shein,R.D., (1979). Epidemiology and plant disease management. Oxford, New York. 427pp.

A Note on Sesame Gall Midge *Sphondylia Sesami* Felt. (Diptera; Cecidomyidae) in the Blue Nile State, Sudan

Eisa Y. Adam[1], Omer Abdallgader[2], Ameer A. Yousif[3]

[1]Damazin Research Station
[2]Gezira Research Station
Agricultural Research Corporation – Crop protection

Abstract— *Sesame gall midge Asphondylia sesami is one of important pest on sesame in the Sudan . A survey was carried out in the Blue Nile State- Sudan, season 2017/2018, the same observation was done in South Kordafan state 2007/2008 for sesame gall midge damage determination on sesame. The damage observed by the maggot feed inside the floral buds and young capsule leading to formation of galls. Three sites was surveyed for sesame gall midge incidence, four farms per site and three unit area per farm were selected, the damage were taken on buds and capsules. it was observed that high infestation was record in late sowing date throughout different sites, the damage which affected on yield was observed on buds and flowers which wither and drop.*

Keywords— Gall, Midge, Bud, Damage, Infestation.

I. INTRODUCTION

Sesame (*Sesamum indicum* Linnaeus) is an important oilseed crop mainly grown in tropics and sub-tropics and major producing countries are India, China, Turkey, Myanmar, Pakistan, Egypt, Sudan, Greece, Venezuela, Argentina, Colombia, Nicaragua, Elsalvador, Mexico and USA. Sesame is a warm weather crop and is often grown under marginal or stressed conditions (Rojeet, 2012). *Sesame indicum* L. is an oil seed crop, with oil contents varying between 40% and 60% according to crop variety. Sesame is a major oil crop produced in Sudan under mechanized and traditional rainfed system of the central clay plains (Gadarif and Blue Nile States) and on clay and qoz lands of western Sudan (Kordofan and Darfur States). Sudan is also one of the largest exporters of sesame ranking second in the world as it exported about 124000 tons annually on average about 18.3% of total worlds exports (Hala, 2010)

The sesame crop attack by many pests: leaf webber and capsule borer (*Antigastra catalaunalis* Duponchel), red hairy caterpillar (*Amsacta albistriga* Walker), horn worm (*Acherontia styx* Westwood), pod bug (*Elasmolomus sordidus* Fabricius) and also the phytoplasma disease phyllody transmitted by *Orosius albicinctus* Distant. (Diraviam, 2014).

Sesame gall midge *Asphondylia sesami* restricted to East Africa and Southern India (Ahuja, *et al*. 2001). The sesame gall midge reported as a major pest from Maharashtra (India). The incidence of the pest is common during flowering period of sesame and number of generation will be increased due to staggered sowing as well as inclusion different verities of sesame (Baskaran, *et. al*. 1997).

Phillips (1977) reported that gall midge *Asphondylia sesami* is one of the main insects pest of sesame in Nigeria. In Sudan the pest was reported by Schmutterer (1969), occurs in the southern Fung (Tozi), in Nuba Mountains (Kadugli, Talodi) and in Equatoria Province (Magwe, Yei).

II. DESCRIPTION

Adult midge about 4 – 5 mm long and pale orange in colour. Legs slender, yellowish – brown. Forewings transparent, dusky and covered by numerous small grayish hairs. Distal part of halters strongly enlarged. Eye dark, dorsal connected. Antennae long, 14 – jointed. Abdomen of female with long protrusible ovipositor (Schmutterer,1969).

III. LIFE CYCLE

The female lays its eggs singly by its needle-like ovipositor in the flowers and buds and flowers of sesame (Schmutterer, 1969). The egg period is 2-4 days; the larval period is about 2-3 weeks with the 4 larval instars. The numbers of larvae observed per bud varied from 3 – 14. The fully fed larvas cut a hole in the bud, drops and pupate in the soil 5 – 7 cm deep or

inside the malformed capsules. The fly emerges from galls in 7-12 days. The total life cycle is completed in 23-27 days (Baskaran, *et. al*. 1997).

IV. DAMAGE, ECONOMIC IMPORTANCE AND MANAGEMENT

Usually a miner pest but occasionally high infestation occurs with resulting considerable crop losses. The irritation caused by feeding of larvae results in drops of flower buds and formation of gall like buds, which do not develop in to seeds capsules. These reduce seed yield by up to 100% in susceptible genotypes and under favourable conditions. Capsule damage due to gall midge of up to 29-34.3% has been reported in Uganda (Ubor, et. al. 2015).

Monitoring of the plants at the time of bud initiation is necessary; use the resistant and tolerant varieties. Early sowing of the crop may prevent heavy damage as the pest usually appears in numbers in the late mid rainy season intercropping with mungbean, pear millet and groundnut. Clip the galls, pick and burn the shed buds. Conserve natural enemies, larval parasites like *Eurytoma sp*., *Baracon hebetor.* Use neem product when necessary as it reduce capsule damage by gall fly.

V. SURVEY CONDUCTED

A survey was carried out in the Blue Nile State season 2017/2018 mainly in the late sown sesame. The damage observed by the maggot feed inside the floral buds and young capsule leading to formation of galls, flower buds become wither and drop, or become twisted and stunted and do not develop in to flower or capsules and the pods were develops completely distorted and contain no seeds.

Three sites was selected (Damazin Experimental farm, East and North El Damazin) was surveyed for sesame gall midge incidence, four farms per site and three unit area per farm were selected, the damage were taken on buds and capsules, Ten plants were taken randomly and total number of buds, capsule, infested buds and infested capsules to determined percentage damage. The same observation was done in South Kordafan state 2007/2008 when the pest were outbreak in that time.

The pest was rear in the lab till adult emerge were keep will and send to Wad Medani, insect collection unit (ARC) for identification.

Through the survey it was observed that high infestation was record in late sowing date throughout different sites, the damage which affected on yield was observed on buds and flowers which wither and drop, Moreover early sown crop escaped the pest attacks with a light infestation on tender plants. The infestation on flowers was high than that on capsules. During the survey there are two genus of the parasitoid was recorded on sesame gall midge pupa and identified as *Eurytoma sp* and *Baracon hebetor*. The results of percentage damage by sesame gall midge was presented in (Figs. 1, 2)

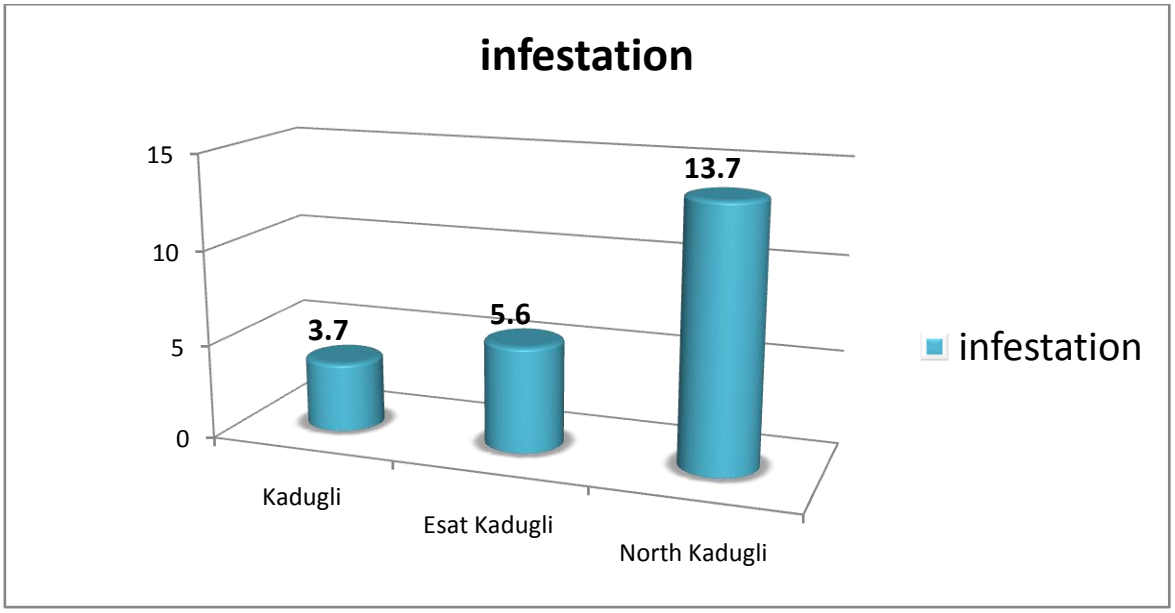


FIGURE 1: Mean percentage of infestation on sesame capsule caused by sesame gall midge in three different location, South Kordofan 2007/2008

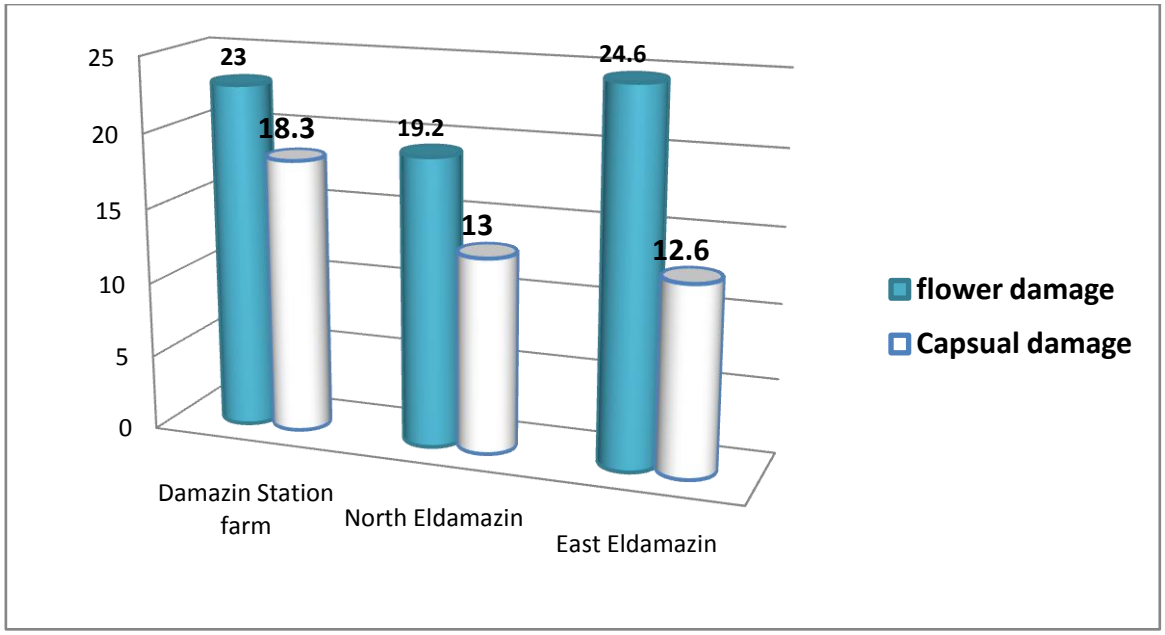


FIGURE 2: Mean percentage of infestation on sesame flower and capsule caused by sesame gall midge in three different location, Blue Nile State, 2016/2017

FIGURE 3: Damage on leaves by sesame gall midge

FIGURE 4: Damage on buds and flowers

FIGURE 5: Damage on capsule and gall formation

FIGURE 6: Sesame gall midge (Adult)

VI. CONCLUSION

It was observed that high infestation was record in late sowing date throughout different sites, the damage which affected on yield was observed on buds and flowers which wither and drop, Moreover early sown crop escaped the pest attacks with a light infestation on tender plants. The infestation on flowers was high than that on capsules.

REFERENCES

[1] Ahuja D. B. and Kalian R. K. , Sesasonal incidence of gall fly, Asphodyli sesame and efficacy of various nee, based formulation, against it on sesame> Indian Journal of entomology, 2001, Vol. 63, No. 4, PP. 409 – 412. ISSN 0367 – 8288.

[2] Baskaran R. K., Mahadevan N. R., Sathiyanadam V. K. and Thangavelu S., Approaches prevention and control of sedame pests. Recent advances in entomological research. Regional Research Station. Tamil. Nadu Agricultural University, 1997, P. 133 – 136.

[3] Diraviam J., Observations of major sesame pests and disease and their possible off-season hosts in Karur district, Tamil Nadu Insect Environment, 2014, Vol. 20(3), P. 95.

[4] Hala Y. A, The Economics of Sesame Production and Marketing in Gadarif and North Kordofan Rainfed Sector - Sudan, A thesis presented to University of Khartoum for fulfillment of the requirement for the Ph.D. Agric. Econ, 2010, P. 1 -5.

[5] Hill D. S, The Major tropical crops of Warmer Climate and Their control, 2008, P. 352.

[6] Rojeet T., Investigation on insect pests of Sesame and their management with special reference to phyllody vector, Thesis submitted to the University of Agricultural Sciences, Dharwad In partial fulfillment of the requirements for the Degree of Master of science (Agriculture) in Agricultural Entomology, 2012, P 2.

[7] Schmutterer H., Pests of crops in Northeast and Central Africa, 1969, pp. 193.

[8] Phillips T. A. Sesame insects pest and disease. Agricultural Note Book, Annual crops. Agriculture, Eastern Region, Nigeria ,1977, PP. 63.

[9] Ubor W., Gibson P., Anyanga W. and Rubaihayo P, Inheritance of resistance to sesame gall midge in Uganda, African Crop Science Journal, 2015, Vol. 23, No. 4, pp. 356.

[10] http://www.eagri.org/eagri50/ENTO331/lecture09/linseed/pdf/lec09.pdf

[11] http://www.jnkvv.nic.in/IPM%20Progect/insect-sesamum.html.

Effect of Chlorine Treated Water on Germination and Growth of Cowpea Cultivars (*Vignaunguiculata* L. Walp)

Olasan, J.O.[1], Zara, M.[2], Atim, H.L.[3]

[1,3]Plant Science and Biotechnology Unit, Federal University of Agriculture, Makurdi. Nigeria
[2]Department of Applied Chemistry, Kaduna Polytechnic, Kaduna

Abstract— *Effects of different water qualities (WQ_1- WQ_5) of varying chlorine contents were tested on the growth and germination of four varieties of cowpea (Vignaunguiculata) IT03k 131-2 (v_1), IT99k -573-1-1(v_2), UAM09 1046-6-1 (v_3) and UAM09 1055-6 (v_4). The experimental design was factored using Completely Randomized Design (CRD) with three (3) replicates for each treatment of the five (5) water qualities for four (4) cowpea varieties. Growth parameters, germination rate, seedling vigor indices and chlorophyll content were measured. The interactions of cowpea variety and water quality had no significant effect on the any growth parameter tested ($p>0.05$). Variety factor was largely insignificant ($p>0.05$) with minor exceptions. Water quality factor had significant effects on all growth parameters of cowpea ($p<0.05$). Germination rate was highest in pond water (no chlorine) but least in disinfected water when10g and 20g of chlorine were applied. Percentage germination recorded the high values of 98.6% and 95.8% in pond and river water respectively. Water-treated plant without additional chlorine had the same germination with river water (95.8%). 10gCl and 20gCl added to disinfected water reduced cowpea germination to 10.1% and 0.5% respectively. Chlorination had significant effects on seedling height from 7 day to 28 day after planting ($p<0.05$). Seedlings treated with disinfected water were the tallest at 28 day after planting (18.1cm) followed by river and pond water (16.2cm and 16.1cm respectively). Heights of seedlings reduced drastically to 0.5cm on addition of 10g and 20g of chlorine. The two best vigour indices were found among seedlings treated with pond water (1186) and disinfected water (1172). Vigour were significantly reduced when seedlings were treated with additional chlorination ($p<0.05$). The same trend was observed in the germination speed indices of seedlings. Shoot and root weight were also reduced by chlorine. Disinfected water +10g of chlorine and disinfected water +20g of chlorine recorded zero weight (0.000g). The highest chlorophyll content was found in the leaf of plant treated with disinfected water (1.799) followed by river water (1.658) and pond water (1.402). No chlorophyll test was conducted on plants treated with additional chlorine as they died off before maturity when treated with DFW+10gcl and DFW+20gcl. As a result, normal disinfection yielded the same result as pond and river water having no significant effect on the growth parameters evaluated. However, additional chlorination (DFW+10gcl and DFW+20gcl) significantly affected the cowpea cultivars ($p<0.05$).Therefore, municipal water treated with chlorine for drinking should be considered safe for irrigating the crop. However, high chlorine concentrations adversely affect the crop and this outcome may also be applicable to other commercially cultivated crops of huge importance to the economy.*

Keywords— Cowpea, Chlorine, Water quality, Growth parameters.

I. INTRODUCTION

Cowpea is of Africa and Asian origin (perrino *et al.*, 1993, Ogunkanmi *et al*., 2005). In Nigeria, it is grown all over the country but with varying sowing dates. The major cultivation centre includes Kano, Katsina, Bauchi, Borno, Sokoto and Niger in the North, Ibadan, Owo, Benin and Asaba in the South (Rachie, 1985).The cowpea (*Vignaunguculata*) is one of several species of the widely cultivated genus Vigna. Four subspecies are recognized, of which three are cultivated (more exist, including textiles pubescens, and sinensis) (Perrino *et al*., 1993). Most cowpeas are grown on the African continents, particularly in Nigeria and Niger which account for 72% of world cowpea production (FAO, 2015). Cowpea are grown

mostly for their edible beans, although the leaves, fresh peas and fresh pea pod can also be consumed, meaning the cowpea can be used as food source before the dried peas are harvested (Ehlers and Hall, 1997).Cowpea is an important source of food for humans in poor arid regions the crop can also be used as feed for Livestock this predominantly occurs in India, where the stock is fed cowpea as forage or fodder (Singh *et al.,* 1997). Cowpea provides a rich source of proteins and calories as well as minerals and vitamins. A cowpea seed can consist of 25% protein and is low in anti-nutritional factors (Rangel, 2003). The diet complements the mainly cereal diet in countries that grow cowpeas as a major food crop (Philips, 2003).

The demand for cowpea production keeps increasing as it is one of the cheapest source of protein and as such the production cannot be narrowed only to seasonal (rainfall) production. The increase in cowpea production also depends on other production practices such as irrigation using other available water qualities (such as pond, river or disinfected domestic water). The roles of water in the seed germination, plant growth and physiological functions cannot be over emphasized (Taylor *et al*., 2007). In the commercial production of cowpea, different types of water from different sources are used in irrigation most of which are disinfected with chlorine. Poor yield may be ignorantly attributed to other factors without prior knowledge of the effect of chlorine content on the general well being of the plant. Water quality varies from source to source. Different sources of water are commonly used by growers to irrigate crops in Nigeria among which are: well water, municipal water and pond water. Chlorination is used to kill certain bacteria and other microbes in tap water as chlorine is highly toxic. In particular, chlorination is used to prevent the spread of water borne diseases such as cholera, dysentery, jaundice, typhoid etc. (EPA, 2014). Disinfection by chlorination can be problematic, in some circumstances. Chlorine can react with naturally occurring organic compounds known as disinfection by products (DBPs). The most common DBPs are trihalomethanes (THMs) and shaloacetic acids (HAAs), which mainly responsible for health hazard (WHO, 2011). This present study was designed to determine the effect of chlorine on germination and growth by comparing chlorinated water with municipal and natural water bodies. The most effective water quality would be recommended for cowpea irrigation.

II. MATERIALS AND METHODS

2.1 Sources of materials

The four varieties of cowpea seeds were obtained from the Molecular Biology Laboratory of University of Agriculture Makurdi. These include: IT03k-131-2 (v_1), IT99k -573-1-1(v_2), UAM09 1046-6-1 (v_3), UAM09 1055-6 (v_4). Four different water qualities used for the experiment were: Disinfected water (chlorinated) WQ_1); River water (WQ_2); Pond water (WQ_3); Disinfected water + 10gcl (WQ_4) and Disinfected water + 20gcl (WQ_5). Disinfected water (chlorinated water) was obtained from the Water Works Department of the University of Agriculture Makurdi. River water was collected from River Benue in Makurdi pond was collected within the university. Chlorine was collected from the quality control department of Consolidated Brewery Plc, Makurdi, Nigeria.

2.2 Elimination of Interfering factors

Soil used for this experiment was steamed sterile at the temperature of 100^{o}C for 45minutes. This was done to avoid interference of organic matter with the chlorine. The soil was analysed at the Advanced Soil Science Laboratory, University of Agriculture Makurdi. Soil particle size distribution was determined by Bouyaucos's Hydrometer using sodium hexametaphosphate (algon) as the dispersing agent. (Bouyouco, 1962).Soil and water pH was determined using pH meter (electrometric method).

2.3 Experimental Design

The design was set up as factorial experiment, using Completely Randomized Design (CRD) with three (3) replicates for each treatment of the five (5) water qualities for four (4) cowpea varieties. This was designed such that V_1-V_4 was each tested

with WQ_1, WQ_2, WQ3, WQ4, and WQ_5. Six (6) cowpea seeds were planted in 60 pots each, filled with 720g of soil, at a depth of 1.5-2cm (Aguoru *et al*., 2015). Germination count began at 5 days after planting. Germination rate was taken at 5, 7, 9, 10 and 13 days after planting and this was estimated using the formular; $\frac{1}{t_n}(\sum Gn)$ (Dniel, 2007).

Where;

$$t_n = total\ time\ taken$$

$$\sum Gn = cummulative\ germination\ count$$

Seedling length was measured at 7, 14, 21 and 28 days after planting using meter rule in cm. Germination capacity was determined by the number of seedlings emerging from the seeds. Percentage germination was determined by

$$G = \frac{n}{N}X100$$

Where;

n= the total number of seeds during germination test; N=number of seeds initiated

Speed of germination was also determined by the equation;

$$\frac{n_1}{t_1} + \frac{n_2}{t_2} + \frac{n_3}{t_3} + \frac{n_4}{t_4} \ldots . \frac{n_5}{t_5}$$

$$GS = \sum(\frac{n_1}{t_1})$$

Where

n=the number of germination seeds on days; t=the number of days during germination period.

The vigor index of seedling was calculated adopting the Baki and Aderson (1973) method. This was expressed as: Seedling Vigor Index (V_1) =germination (%) X seedling length (cm)

i.e.

SV_1= [SL (cm) X G).

Wet shoot and root weight were measured using digital weighing balance (Sekav^{TM}). Chlorophyll test was carried out using spectrophotometer at the wave length of 500nm Chlorophyll extraction followed standard protocol outlined by the Association of Official Analytical Chemists (AOAC, 1984). Chlorophyll test was carried out using spectrophotometer at the wave length of 500nm. Data obtained were subjected to analysis of variance (ANOVA).

III. RESULTS AND DISCUSSION

The effect of chlorinated water on germination and growth of different cowpea cultivars has been successfully investigated using different levels of chlorine. Cowpea varieties only had significant effect ($p \leq 0.05$) on the germination rate at five (5) days after planting and shoot weight per plant. Varieties did not affect other growth parameters measured. Varietal performance shows that germination at 5 days after planting was most significant in the UAM091046-6-1 variety while differences in shoot weight was caused by UAM091055-6 followed by UAM091046-6-1 varieties (table 2). The minor differences observed in the germination rate and shoot weight among cowpea varieties could have genetic basis (Ogunkanmi *et al*., 2005). In the present findings, since the interactions of water quality and variety had no significant effect on the any growth parameter tested ($p>0.05$), varietal differences alone is negligible.

TABLE 1
ANALYSIS OF VARIANCE FOR THE DIFFERENT PARAMETERS MEASURED

Source of variance	Ger @ 5DAP	%Ger	SLH @ 7 DAP (cm)	SLH @ 14 DAP (cm)	SLH @ 21 DAP (cm)	SLH @ 28 DAP (cm)	AVSLH (cm)	SLV INDEX	Ger index	Ger @ 28 days	RW/PLT (g)	SW/PLT (g)
Variety	1.667*	84.579^{ns}	0.015^{ns}	1.078^{ns}	6.752^{ns}	2.493^{ns}	1.421^{ns}	11102.740^{ns}	0.378^{ns}	1.706^{ns}	0.0727^{ns}	0.531*
Water quality	65.900**	3280.296**	75.927**	365.715**	553.350**	363.038**	417.236**	4409284.840**	32.525**	56.983**	2.3013**	9.675**
Water quality X Variety	1.222^{ns}	46.295^{ns}	0.277^{ns}	1.039^{ns}	3.266^{ns}	1.215^{ns}	0.758^{ns}	5577.060^{ns}	0.315^{ns}	0.758^{ns}	0.1091^{ns}	0.271^{ns}

**** = significance at 0.05 (5%) and ** = are highly significance at 0.01 (1%).***
Ger @ 5DAP= Germination rate at five (5) days after planting
%Ger=Percentage germination; SLH @ 7 DAP=Seedling height at 7 days after planting and so on
SLH @ 28 DAP= Germination capacity at 28 days after planting; AVSLH=Average seedling height
SLV INDEX= Seedling vigor index; Ger index=Germination speed index
RW/PLT (g) = Root weight per planting (g); SW/PLT (g) = Shoot weight per plant (g)

TABLE 2
MEAN PERFORMANCE OF COWPEA VARIETIES

Variety	Ger @ 5DAP	%Ger	SLH @ 7 DAP (cm)	SLH @ 14 DAP (cm)	SLH @ 21 DAP (cm)	SLH @ 28 DAP (cm)	AVSLH (cm)	SLV INDEX	Ger index	Ger @ 28 days	RW/PLT (g)	SW/PLT (g)
UAM091046-6-1	3.533a	63.467a	3.267a	7.133a	8.300a	10.533a	7.210a	686.68a	2.397a	3.266a	0.909a	1.686a
IT99k-573-1-1	2.866b	57.978a	3.220a	6.833a	8.726a	10.667a	6.67a	627.87a	2.163a	3.066a	1.0689a	1.473^{ba}
IT03k-131-2	2.866b	60.167a	3.220a	7.166a	8.106a	10.133a	7.345a	684.08a	2.477a	2.66a	0.949a	1.408^{ba}
UAM091055-6	2.866b	59.056a	3.220a	6.600a	7.140a	9.766a	7.291a	663.78a	2.173a	2.66a	0.9421a	1.226^{b}
Mean	3.033	60.16%	3.221	6.933	8.068	10.275	0.458	665.645	2.303	2.88	0.458	1.4667
LSD	0.539	0.39	0.657	1.454	1.815	1.174	1.066	113.27	0.37	0.803	0.838	0.296

Legend:
Value followed by the same alphabet are not statistically significance at $p \leq 0.05$
LSD= Least Significant Difference

TABLE 3
MEAN SEPARATION OF WATER QUALITY

Water variety	Ger @ 5DAP	%Ger	SLH @ 7 DAP	SLH @ 14 DAP	SLH @ 21 DAP	SLH @ 28 DAP	AVSLH	SLV INDEX	Ger index	Ger C @ 28 days	RW/PLT (g)	SW/PLT (g)
Pd	5.333a	98.611a	5.566a	12.208a	14.300a	16.083b	12.039a	1185.97a	3.892a	4.333a	1.4316a	2.033b
RVW	4.500b	95.833a	4.458[b]	9.166b	9.800b	16.208b	9.908b	950.03b	3.325b	4.416a	1.2498[ba]	1.925b
DFW	4.333b	95.833a	5.083[ba]	11.2500a	14.400a	18.083a	12.204a	1172.92a	3.256b	4.666a	1.1542[b]	2.375a
DFW+10gcl	0.500c	10.056b	0.500c	1.541c	1.342c	0.500c	1.002c	18.18c	0.539c	0.500b	0.500c	0.000c
DFW+20gcl	0.500c	**0.500c**	0.500c	0.500c	0.500c	0.500c	0.500c	0.500c	0.500c	0.500b	0.500c	0.500c
Mean	3.033	60.16%	3.221	6.933	8.068	10.275	0.458	665.645	2.303	2.88	0.458	1.4667
LSD	0.603	6.390	0.735	1.625	2.03	1.313	0.937	126.64	0.413	0.898	0.2537	0.331

Legend:
Pd = Pond water; Rvw = River water; Dfw = Disinfected water
Dfw + 10gCl = Disinfected +10g of Chlorine
Dfw + 20gCl = Disinfected +20g of Chlorine
Value followed by the same alphabet are not statistically significance at p > 0.05

TABLE 4
CHLOROPHYLL TEST USING 500NM WAVELENGTH

WQ_1(Disinfected water)	WQ_2 (River water)	WQ_3 (Pond water)	WQ_4 (disinfected +10g Cl)	WQ_5 (disinfected +20g Cl)
1.799	1.658	1.402	0	0

As presented in table 3, water quality had significant effects on all growth parameters of cowpea ($p<0.05$). Germination rate was highest in pond water (no chlorine) but least in disinfected water when10g and 20g of chlorine were applied. Percentage germination recorded the high values of 98.6% and 95.8% in pond and river water respectively. Disinfected water- treated plant without additional chlorine had the same germination with river water (95.8%). 10gCl and 20gCl added to disinfected water reduced cowpea germination to 10.1% and 0.5% respectively (figure 1). Chlorination had significant effects on seedling height from 7 day to 28 day after planting ($p<0.05$). For instance, seedlings treated disinfected water were the tallest at 28 day after planting (18.1cm) followed by river and pond water (16.2 cm and 16.1cm respectively). Heights of seedlings reduced drastically to 0.5cm on addition of 10g and 20g of chlorine to disinfected water (figure 2).

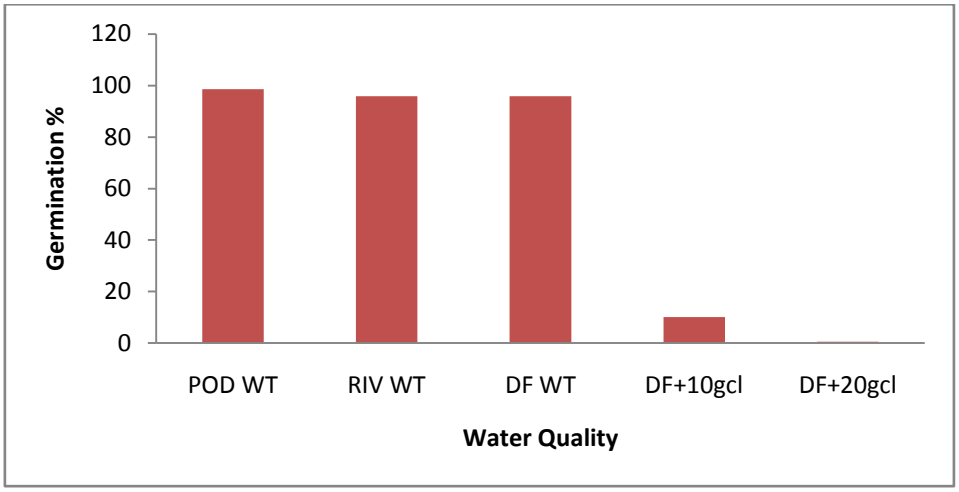


FIGURE 1: Influence of chlorine concentration on germination

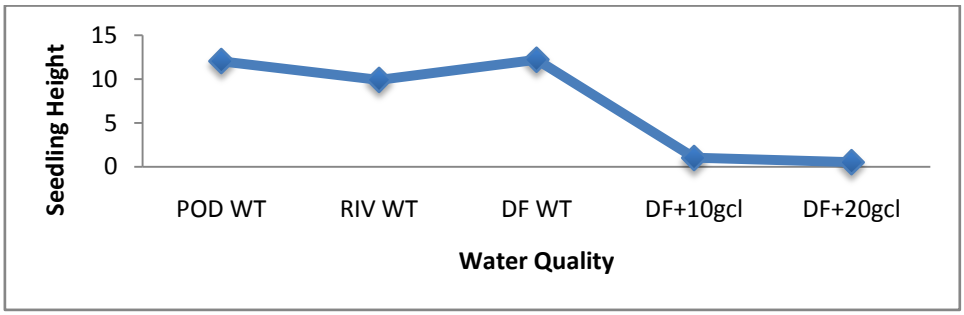


FIGURE 2: Influence of chlorine concentration in seedling height

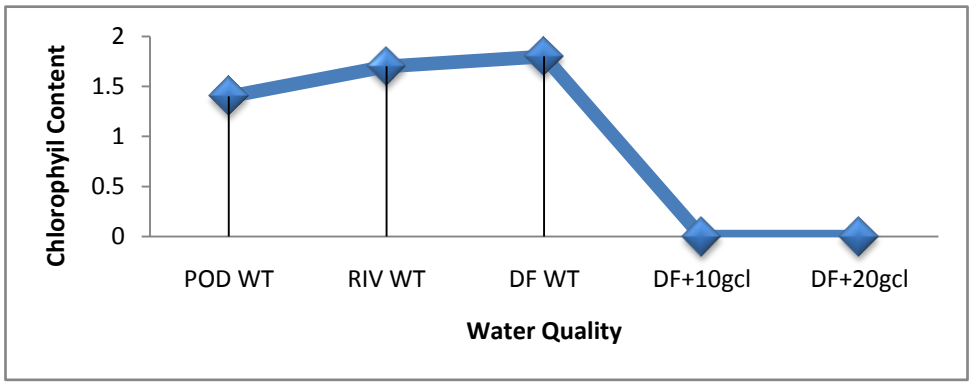


FIGURE 3: Effect of chlorine concentration on chlorophyll

This result could be attributed to the differences in the acidity alkalinity and salt level affecting the pH and overall quality of water treatments. This finding is in line with the report of Bukiv *et al.* (2007) who also found significant differences in seedling growth in white clover and alfalfa genotypes under different pH values of water. However, interaction effect of water quality and variety had no significant effect on any growth parameter tested ($p>0.05$). The present study also aligns with the view of Villagra (1997) that salinity affects seed germination and plant growth. According to Phulari (2013), chlorinated water has inhibitory effect on plants vital process during seed germination. High concentration of chlorine was

earlier reported to affect seedling growth and radical length of cowpea (Phulari, 2013).It can be deduced that high concentration of chemical compounds released into the environment may affect the overall plant performances (Aguoru *et al*., 2008, 2015a, 2015b; Egbutah *et al*., 2015). The metabolic activities of all living organisms are catalyzed by enzymes whose functionality depends on water and its quality (Taylor *et al*., 2007). Water is a major limiting factor affecting all life forms, hence its quality assessment is essential (Aguoru and Katsa, 2009; Maduka *et al*., 2014).

The best vigour indices were found among seedlings treated with pond water (1186) and disinfected water (1172). Vigour were significantly reduced when seedlings were treated with additional chlorination to disinfected water ($p<0.05$). The same trend was observed in the germination speed indices of seedlings. Shoot and root weight were also reduced by chlorine. Disinfected water +10g of chlorine and disinfected water +20g of chlorine recorded zero weight (0.000g). According to Olasan *et al*. (2017), seedling vigour is a physiological response attributed to genetic differences. The authors found out that wet biomass of groundnut (*Arachis hypogaea*) in relation to moisture content of the seedling are a major determinant of its overall vigour. In addition, seedling vigour could be affected by external influences (environment) as they interact with genes. This view corroborates the present investigation. As a result, normal disinfection yielded the same result as pond and river water having no significant effect on the growth parameters evaluated. However, additional chlorination (DFW+10gcl and DFW+20gcl) significantly affected the cowpea cultivars ($p<0.05$).

As given in table 4, the highest chlorophyll content was found in the leaf of plant treated with disinfected water (1.799) followed by river water (1.658) and pond water (1.402). No chlorophyll test was conducted on plants treated with additional chlorine as they died off before maturity when treated with DFW+10gcl and DFW+20gcl (figure 3). In the work of Cayanan *et al.* (2008), normal level of chlorine treatment did not affect leaf chlorophyll content after 11weeks of growth. Frink and Bugbee (1987) reported phytotoxic effects of chlorine on several greenhouse crops and their free chlorine thresholds which range from $2.mg.L^{-1}$ to $77mg.l^{-1}$. In the present study, the effect of excess chlorine on chlorophyll synthesis can be seen as inhibitory. This might have affected the photosynthetic apparatus with resultant effect on the level of substrate (sugar) needed in ATP production to power metabolic activities (Taylor *et al*., 2007). Hence, the affected plants failed to survive to maturity.

IV. CONCLUSION

In conclusion, this present study has revealed that the low level of chlorine in municipal water supply does not affect cowpea germination and growth with 95% confidence limit. Therefore, municipal water treated with chlorine for drinking should be considered safe for irrigating the crop. This may also be applicable to other crops. However, high chlorine concentrations adversely affect the crop. In addition more highly demanded crops should be tested under greenhouse and field conditions and at lower concentration of chlorine other than the level used in this experiment.

REFERENCES

[1] Aguoru, C.U., Amuzie, U.F., Ogbadu, G.H. and Odiaka, N. (2008).Effects of different combinations of auxin (NAA) and cytokinin (BAP) on growth of (*Ipomoea batatas* L) sweet potato *in-vitro*.*International Science Research Journal*, 1(2): 90-92.

[2] Aguoru, C.U. and Katsa, M.M. (2009).Quality assessment of drinking water from different sources in Lafia, Nassarawa State, Nigeria.*International Journal of Natural and Applied Sciences*, 5(2):150-156.

[3] Aguoru, C.U., Hassan, A. and Olasan, J.O. (2015a). Comparative Study of the Efficacy of Cowdung and N.P.K Fertilizer on the Growth of Cowpea (*Vignaunguiculata*), Soyabean (*Glycine max*), Tomato (*Lycopersicumesculentum*) and Pepper (*Capsicum frutescens*) in North Central Nigeria. *International Journal of Pure and Applied Sciences and Technology*, 28(1): 24-33.

[4] Aguoru, C.U., Ajogu, P.A.J. and Olasan, J.O. (2015b). Studies on the Environmental Impact of the Activities of Rice Mills within Makurdi metropolis, North Central Nigeria.*International Journal of Renewable and Environmental Sciences*, 3(4): 1-7.

[5] AOAC. (1984). Official Methods of Analysis, Arlington, V.A. (Ed.), 14thEdn.,Association of Official Analytical Chemists.

[6] Bouyouco, G. J. (1962). Hydrometer methods improved for making particle size analysis of soils. *Soils Science of America Proceeding*, 26: 464 – 465.

[7] Bukiv, Gordana, SondjaGrljusic, VlatkaRozman, S. Popovic, V. Lucin (2007).Infleucne of temperature and pH value on germination of alfalfa genotypes, BooL of Abstracts of 42nd Croatian and 2nd International Symposium on Agriculture, Opatija – February 13 – 16, 2007, 85 – 86.

[8] Cayanan, D. F., Zheng, Y., Chong, C., Zhang, P., Graham, T. Llewellyn, J., and Dixon, M. (2008b).sensitivity of five container grown nursery species to chlorine in overhead irrigation water.*Hort Science*, 43: 1882 – 1887.

[9] Egbutah, E.U., Obasi, M.O., Kalu, B.A. and Aguoru, C.U. (2015). Correlations among seed yield and yield attributes in roselle (*Hibiscus sabdariffa* L.) as influenced by previous intercrop and nitrogen fertilization. *Discourse Journal of Agriculture and Food Sciences*, 3(3): 59-64

[10] Ehlers, J. D. and Hall, A. E. (1997). Cowpea (Vignaunguculata L. Walp) Field Crop Res. 53, 187 – 204.

[11] FAO (2015).FAOSTAT gateway. Retrieved from: www.fao.org

[12] Frink, R., Bughbee, G. J. (1987). Response of potted plants and vegetable Seedlings to Chlorinated Water.*Hort Science*, 22: 581 – 583.

[13] Maduka, H.C.C., Okpogba, A.N., Ogueche, Dike, C.C., Ugwu, C.E., Aguoru, C.U.,Adejor, J., Abah., E.L., Maduka, M.A., Gadaka, M.A. and Okonkwo, C.O. (2014). Assessment of some commonly bottled and sachet water in University of Agriculture Makurdi Benue State Nigeria by microbiological investigation. *International Journal of AppliedScience and Technology*, 23(2): 1-6.

[14] Mengel, K., E. A. Kirkby, (1987). Principles of Plant Nutrtion, International Potash Institute, Bern.

[15] Ogunkanm, L. A., Taiwo, A., Mogaji, O. L., Awobodede, A., Eziashi, E. E. and Ogundipe, O. T. (2005). Assessment of Genetic Diversity Among Cultivated cowpea (*Vignaunguiculata* L. Walp) cultivars from a range of localities across west Africa using agronomic traits. *Journal Sci. Res. Dev*. 10: 111 – 118.

[16] Olasan, J.O., Aguoru, C.U., Ugbaa, M.S., Ekefan, E.J. (2017a). Physiological Characterization of Groundnut (*Arachis hypogaea* L.) Seedlings using Vigour and Performance Indices.*Proceedings of the Genetic Society of Nigeria 41st Annual Conference, University of Agriculture Makurdi*, Pp84-91.

[17] Perrino, P., Laghetti, G., Spagnole-Hizeuli, P. and Monti, L. M. (1993).Diversification of cowpea in the Mediterranean and other centres of cultivation.Genetic*Resoruces and Crop Evolution*, 40: 121 – 132.

[18] Philips, R. D., McWatters, K. H., Chinnan, M. S., Hung, Y. C., Nnanyelugo, D. and Enwere, J. (2003).Utilization nof cowpeas for human food.*Field Crops Res*. 82: 193 – 213.

[19] Phulari, S. (2013).Effect of chlorine water on some vital process of LPD Vignaunguiculata L. (Cowpea).*Indian Streams Research Journal*, 13(7).

[20] Rachie, K. O. (1985). Introduction. In: Singh and K. O. Rachie (Eds), cowpea research production and utilization Wiley, Chicester, England P. xxi – xxviii.

[21] Rangel, A., Domont, G. B., Pedrosa, C. and Ferreira, S. T. (2003). Functional properties of purified vialins from cowpea (*Vignaunguiculata*) and pea (*Pisumsativum*) and cowpea protein isolate. *Journal of Agricultural and Food Chemisty*, 51: 5792 – 5797.

[22] Singh, B. B., Mohan, D. R., Dashiell, K. E. and Jackal, L. E. N. (1997).Advances in cowapea in the Mediterranean and other centres of cultivation.*Genetic Resources and Crop Evolution*, 40: 121, 132.

[23] Taylor, D.J., Green, N.P.O. and Stout, G.W. (2007).Biochemicals of Life.*Biological Sciences*.6th*ed*. 765pp.

[24] Villagra, P. E. (1997). Germination of prosopisargentian and prosopisalpataco seeds under saline conditions. *Journal of Arid Environment* 37: 261 – 267.

[25] Welbaum and Bradford (1988).Changes in seed quality during fruit maturation. Physiological and biochemical processes regulating seed germination. *Seed Science Research*, 8: 123 – 135.

[26] WHO (2011).World Health Organization.Chemicals and Health. Retrieved from:www.who.org

Monitoring the Impact of Surface Water Flooding on Groundwater Quality around Nyabarongo River in Rwanda

Prosper Manikuze[1], Jean D'Amour Nsekanabanga[2], Abias Maniragaba[3]

Department of Environmental studies, University of Lay Adventists of Kigali (UNILAK), Kigali-Rwanda

***Abstract*—** *Rwanda exhibits a climate characterized by two rainy seasons in which erosions and inundations are likely to occur and cause the flooding of rivers which threaten the quality of waters. Most of rivers are connected hydrologically with groundwater aquifers which allow the recharge. This study aimed to monitor the impact of surface water flooding on groundwater quality around Nyabarongo River in Rwanda. Two parameters namely turbidity and color which are directly influenced by flooding of water bodies were monitored between January 2017 and June 2020. Laboratory analyses for turbidity on an hourly and color on a monthly basis were conducted at Nzove Water Treatment Plant Laboratory and the computed monthly average values were used. The laboratory results for surface water and groundwater were compared through graphical presentation which indicated that there is a relationship between the change in quality of both waters due to the fact that the trends in variation of the quality of both waters correlate in the same periods or groundwater quality changes similarly just a little bit after surface water quality has changed. This observation has led to the conclusion that the changes in water quality of Nyabarongo River which is mainly exacerbated by the flooding of March to May and October to December rainy seasons affect the quality changes of the groundwater recharged by this river. This finding indicates the need and urgency of implementation of Nyabarongo catchment rehabilitation and management plans.*

Keywords — Floods, groundwater recharge, impact, Nyabarongo River, water quality change.

I. INTRODUCTION

Rwanda is located within the equatorial belt with a modified humid climate including rainy forest and Savannah types. The rainfall characteristics for Rwanda are known to exhibit large temporal and spatial variation due to varied topography and existence of large water bodies throughout the country as well as the influence of climate change [10]. However, two rainy seasons are generally distinguishable, one centered on March – May and the other around October – December [17]. The existing literature indicate that temporal variability of the rainfall in terms of intensity and frequency in some occasions has resulted in extreme events such as the floods and frequent droughts that have far reaching socio-economic impacts to the country and water resources in particular used to get increasingly polluted [17] and Meteo-Rwanda official website. Water resources in Rwanda occupy a total of 135,000 ha or 8% of the country's surface area. These include 101 lakes (1,495 km^2), 861 rivers totaling 6,462 km and a network of disconnected wetlands [17] while ground Water accounts for 4.554 billion m^3, Rainfall Water for 27.5 billion m^3 per annum, Ground water recharge being 4.5 billion m^3 per annum, Total renewable water is 6.8 billion m^3 per annum while Renewable water availability per capita is 670 m^3 and artificial water storage is 2.5 m^3 [10], [15], [17].

Studies indicated that groundwater aquifer around Nyabarongo river is generally shallow occurring between 3-5m and 12m, with a thickness of at most 8m and is recharged by the river [1]; Nyabarongo floodplain gets completely inundated during the rainy season where the floodwater rise can completely submerge the river, the boreholes and wetland in general, the Nyabarongo River channel is not permanent; thus it significantly changes meanders across its entire flood plain. This has resulted in some boreholes being washed away and lost due to erosion as a result of river encroachment due to river erosion [1], [12] and [14] . This study has monitored the changes in turbidity and color of both surface and groundwater between 2017 and 2020 with a target to examine whether there is a relationship in the changes of both categories of water quality. It is expected that the findings will provide decision makers in water resources management and water supply a baseline information for further mitigation actions.

II. MATERIAL AND METHODS

2.1 Description of the study area

Nyabarongo is the longest river in Rwanda as it touches all the provinces along its flow pathway where it serve water for different purposes such as irrigation, hydropower production, habitat for biodiversity, water treatment and supply, recharging

the surrounding groundwater aquifer. The study area is the part of the river in the Nyabarongo wetland located in Kigali city, Nyarugenge district, Kigali sector, which is currently serving both surface and ground raw water for three Water Treatment Plants (WTP) constructed in the in the area and supplying water to Kigali city and its peri-urban areas. Surface water is conveyed for treatment by two intakes while groundwater intakes consist of 31 boreholes which are designed to abstract underground water and collect in a 600mm diameter pipeline towards another separate WTP as illustrated on Fig.1. As the area is a wetland which has its upstream area of hilly and sloppy prone to erosion and sediments loads together with its proximity to the densely populated Kigali city, it has been undergoing flooding during rainy seasons which used to last for many weeks as the recent flooding of the area in April 2020 lasted around two months [1], [12], [14].

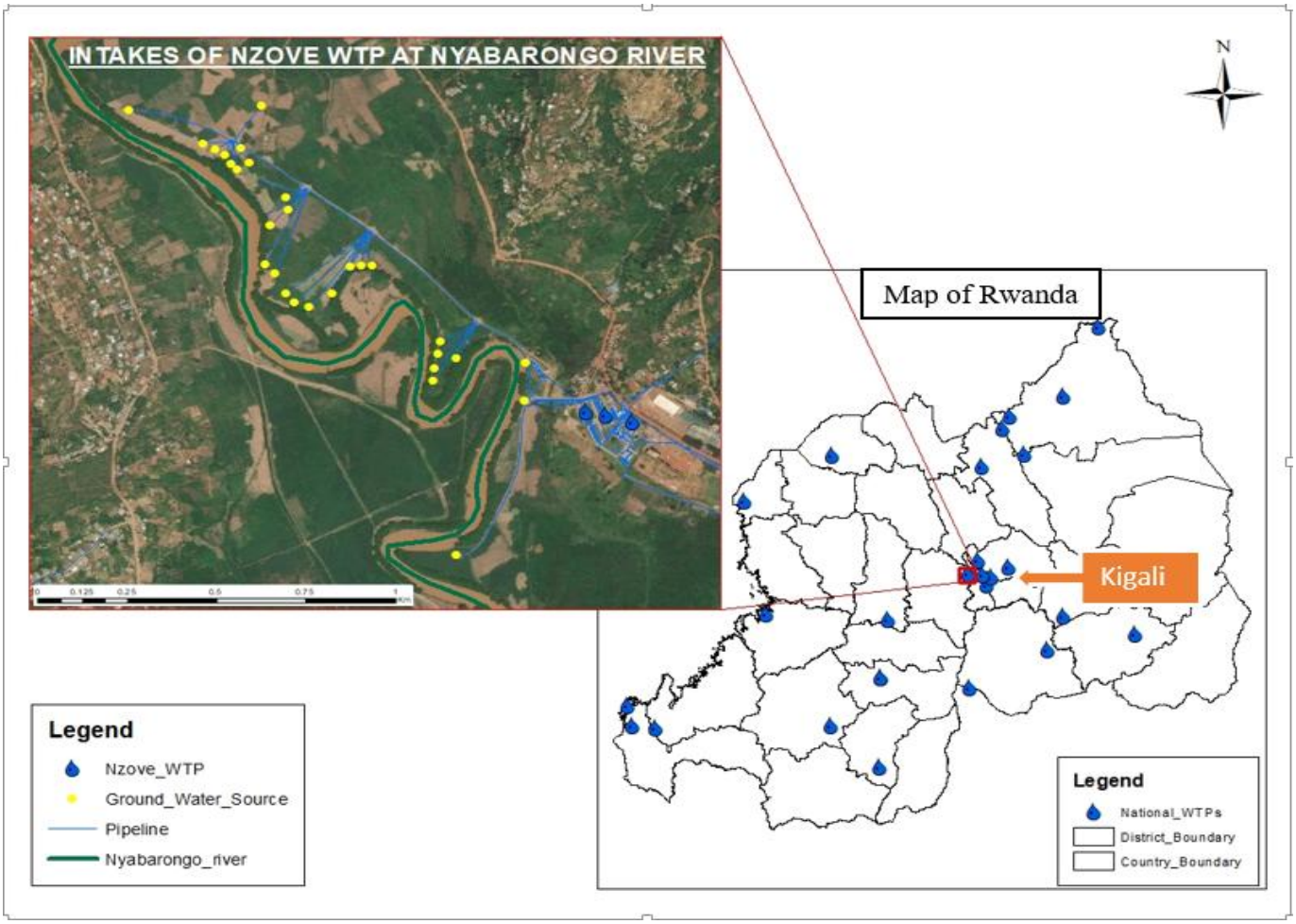


FIGURE 1: Map indicating the location of study area on Rwanda map, the river and groundwater in the study area

2.2 Sampling Techniques

The three parameters that were used in this study namely turbidity, Color and Total Coliform have been chosen based on the fact that they are directly linked to processes such erosion, inundations and floods in water as a route of transportation of sediments, soils and other substances washed in different parts that cause turbidity and color of water to change immediately [10], [12] and [14]. Total Coliform may be caused by entry of soil or organic matter into the water or by conditions suitable for the growth of other types of coliform. In the laboratory total coliforms are grown in or on a medium containing lactose, at a temperature of 35 or 37 °C. They are provisionally identified by the production of acid and gas from the fermentation of lactose. Samples were taken on an hourly basis for turbidity and color and on a monthly basis for total coliform [6] and [7].

Turbidity was measured in situ while for other parameters sample collection was carried out in accordance with the Standard Methods procedures [7]. Samples were collected in sterile 500-ml polyethylene sampling bottles containing 10% sodium thiosulfate and brought to laboratory for analyzed. Working conditions were carefully selected and strict measures were

adhered in avoiding contamination of samples during sampling; handling and storage and all samples were analyzed within 24hours after sampling [4].

2.3 Laboratory analyses

The measurement of turbidity was performed with Nephelometer in compliance with EPA standard for determining turbidity in drinking, ground, surface, waste, and seawater samples and the following steps shall be followed: Preparation of standard solution from formazine which appeared to be more reproducible than other types of standards, a synthetic polymer chosen for its consistency, calibrate the meter with standard cuvettes, fill a cuvette with your sample, clean the outside of the cuvette and if working with samples with very low turbidity, use silicone oil on the outside of the cuvette, place the cuvette inside the meter and take the reading [7].

This method is based upon a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension. The higher the intensity of scattered light, the higher the turbidity. A primary standard suspension is used to calibrate the instrument. A secondary standard suspension is used as a daily calibration check and is monitored periodically for deterioration using one of the primary standards. Formazine polymer is used as a primary turbidity suspension for water because it is previously used for turbidity analysis [4].

The Color was measured using Platinum-Cobalt Standard Method (HACH 8025). A 200ml of sample was placed into 400ml beaker and filtered using 0.45 micron membrane filter. The blank was prepared by pouring 10ml of filtered de-ionized water and placed into the cell holder. Next the 10ml sample was measured using HACH DR 6000 UV-VIS spectrophotometer and the results were taken in Pt/Co (HACH) [7].

In order to measure total coliform**,** MI broth and TSA were prepared. Plates are made ahead of time and stored in the refrigerator, remove them and allow them to warm to room temperature. Label the bottom of the MI broth plates with the sample number/identification and the volume of sample to be analyzed. Using a flamed forceps, place a membrane filter, grid-side up, on the porous plate of the filter base. Attach the funnel to the base of the filter unit so that the membrane filter is now located between the funnel and the base. Put approximately 30 mL of sterile dilution water in the bottom of the funnel and shake vigorously, add 100mL of the sample then. Invert the broth petri dish, and incubate the plate at 35°C for 24 hours. Expose each MI plate to long wave ultraviolet light (366 nm), and count all fluorescent colonies [blue/green fluorescent E. coli, blue/white fluorescent TC other than E. coli, and blue/green with fluorescent edges (also E. coli)] and then calculate the number of colonies using the formula below [4] and [7].

$$\mathrm{TC/100mL} = \frac{\text{Number of fluorescent colonies} + \text{Number of blue, non} - \text{fluorescent colonies (if any)}}{\text{Volume of sample filtered (mL)}} \times 100$$

III. RESULTS AND DISCUSSION

This section presents the results from laboratory analyses in Tables 1, 2 and 3 and the values for all parameters are high during the rainy seasons of March to April and from October to December for surface water of Nyabarongo River. The water from groundwater aquifer recharged by Nyabarongo river also indicate high values in the same period as surface water or a little bit later than the period of increase for surface water. In fact, the historical records and the available literature about the area indicate that Nyabarongo wetland in which the river flows and the groundwater aquifer is located, has been experiencing from the past fifty years heavy floods, inundations and river overflow as a result intensive rainfalls associated with factors like the vulnerability to landslides of the sloppy upstream of the study area and its proximity to Kigali city that exacerbate erosion and wash different sediments, soils and other substances to the river and causing the analyzed parameters to rise. The graphical presentations of the results helped to assess and compare the trend in variation of water quality of groundwater with respect to surface water and the graphs indicate a correlation in variation which has led the authors to detect a relationship between the changes in quality for the two types of waters [10], [12] and [13].

3.1 General presentation of the laboratory analysis results

TABLE 1
RESULTS OF LABORATORY ANALYSIS OF TURBIDITY FOR SURFACE AND GROUND WATER FROM JAN. 2017 TO JUNE 2020

Period	Ground	Surface	Period	Ground	Surface	Period	Ground	Surface	Period	Ground	Surface
Jan-17	8.3	3450	Jan-18	20.1	4200	Jan-19	11.2	6520	Jan-20	18.5	1850
Feb-17	10.2	2440	Feb-18	54.6	5500	Feb-19	10.4	945	Feb-20	17.9	1560
Mar-17	7.2	10500	Mar-18	17.8	14200	Mar-19	17.6	4500	Mar-20	19.3	2415
Apr-17	13.5	14440	Apr-18	45.1	14140	Apr-19	35.4	5100	Apr-20	24.9	6370
May-17	15.4	3920	May-18	36.3	2540	May-19	24.4	3970	May-20	25.6	11250
Jun-17	14.7	1530	Jun-18	32.3	2250	Jun-19	17.9	1640	Jun-20	11.3	14840
Jul-17	6.5	260	Jul-18	14.8	1480	Jul-19	13.8	432			
Aug-17	5.62	451	Aug-18	13	929	Aug-19	9.34	230			
Sep-17	65.3	6530	Sep-18	14.7	1620	Sep-19	9.18	3240			
Oct-17	45.6	4560	Oct-18	14.2	4250	Oct-19	12.7	4560			
Nov-17	20.7	2250	Nov-18	7.2	13450	Nov-19	14.5	3380			
Dec-17	18.4	1820	Dec-18	6.26	18580	Dec-19	20.2	4200			
Unit: Nephelometric Turbidity Unit (NTU) ; Standard Limit for turbidity as per Rwandan standard RS EAC 12-2018: **5 NTU**											

TABLE 2
RESULTS OF LABORATORY ANALYSIS OF COLOR FOR SURFACE AND GROUND WATER FROM JAN. 2017 TO JUNE 2020.

Period	Ground	Surface	Period	Ground	Surface	Period	Ground	Surface	Period	Ground	Surface
Jan-17	105	7360	Jan-18	315	10350	Jan-19	52	3920	Jan-20	400	10510
Feb-17	173	9200	Feb-18	179	10798	Feb-19	39	3920	Feb-20	324	4192
Mar-17	349	8440	Mar-18	940	9550	Mar-19	65	12500	Mar-20	420	3752
Apr-17	480	12765	Apr-18	733	28150	Apr-19	120	12500	Apr-20	1240	13825
May-17	620	15340	May-18	490	10350	May-19	98	2987	May-20	663	13760
Jun-17	96	5284	Jun-18	403	10350	Jun-19	628	3850	Jun-20	540	10570
Jul-17	81	3166	Jul-18	271	1070	Jul-19	76	2013			
Aug-17	76	2570	Aug-18	163	1023	Aug-19	213	23150			
Sep-17	83	1080	Sep-18	102	9850	Sep-19	181	23150			
Oct-17	123	5390	Oct-18	89	14390	Oct-19	102	13300			
Nov-17	157	7990	Nov-18	41	2844	Nov-19	663	3380			
Dec-17	238	8700	Dec-18	41	2844	Dec-19	470	7340			
Unit: Platinum/Cobalt (Pt/Co) ; Standard Limit for turbidity as per Rwandan standard RS EAC 12-2018: **15 Pt/Co**											

TABLE 3
RESULTS OF LABORATORY ANALYSIS OF TOTAL COLIFORM FOR SURFACE AND GROUND WATER FROM JAN. 2017 TO JUNE 2020

Period	Ground	Surface	Period	Ground	Surface	Period	Ground	Surface	Period	Ground	Surface
Jan-17	4.4	93670	Jan-18	6	258600	Jan-19	482	127380	Jan-20	11	22615
Feb-17	16	14540	Feb-18	6	48840	Feb-19	103	241960	Feb-20	244	14230
Mar-17	28	72150	Mar-18	305	14366	Mar-19	6.3	173290	Mar-20	298	24965
Apr-17	44	77010	Apr-18	554	39500	Apr-19	5.2	48810	Apr-20	344	377660
May-17	35	52290	May-18	605	43000	May-19	5.2	40010	May-20	6.3	102400
Jun-17	5	47890	Jun-18	12	43000	Jun-19	7.4	35790	Jun-20	7.7	64300
Jul-17	3	29090	Jul-18	8	43000	Jul-19	6.9	312420			
Aug-17	5	30760	Aug-18	7.5	10900	Aug-19	9	24190			
Sep-17	11	20000	Sep-18	23	84400	Sep-19	6.3	48390			
Oct-17	130	73300	Oct-18	958	193650	Oct-19	6.3	87240			
Nov-17	80	135700	Nov-18	345	32678	Nov-19	9	164780			
Dec-17	55	276000	Dec-18	618	111990	Dec-19	12.4	164780			
Unit: Most probable number (MPN) ; Standard Limit for turbidity as per Rwandan standard RS EAC 12-2018: **Absent**											

3.2 The similarity of trends in change in groundwater quality with respect to surface water

The graphs presented under this subsection serve for a comparison in the trend of variation in the analyzed parameters with respect to water from Nyabarongo River. In all cases similarity in trends indicate a relationship between both water qualities.

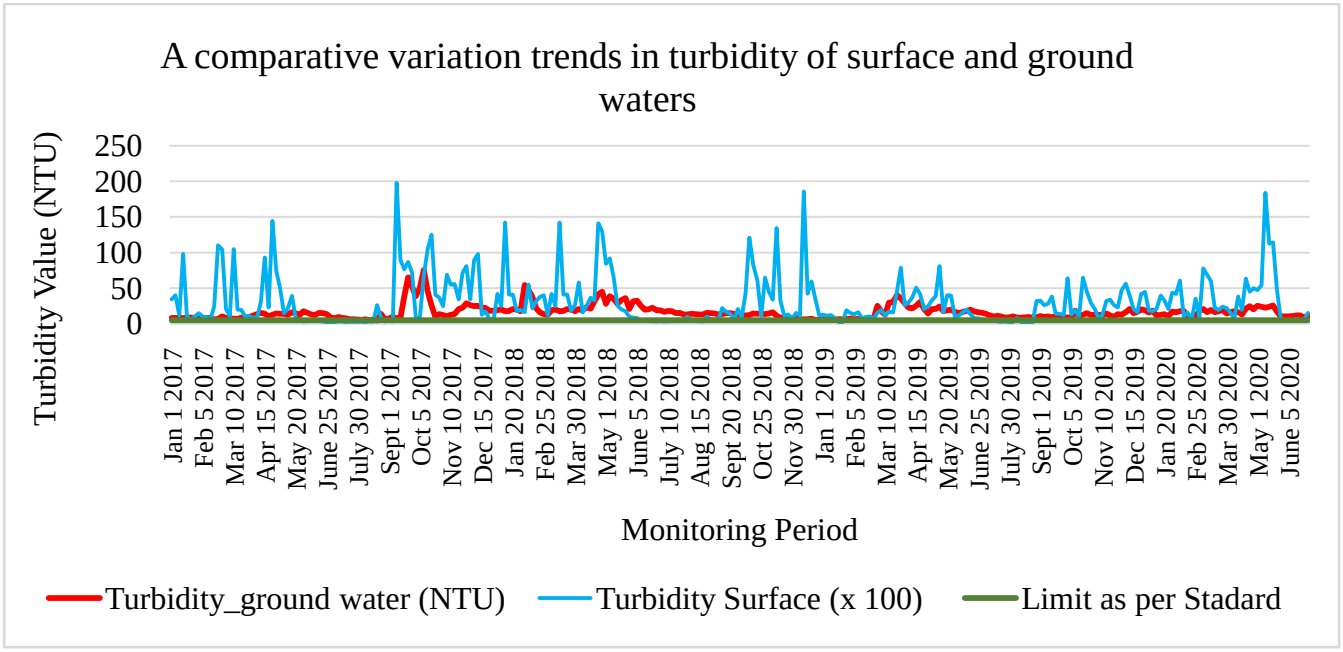


FIGURE 2: Plotted Turidity variations of surface and groundwater for Nyabarongo river and its groundwater aquifer respectively

The trend illustrated in Fig.2 for variation indicated that there is a relationship between surface water quality variation and groundwater. The maximum turbidity values were recorded for both cases in rain seasons or the values for ground water tend to rise a bit after the period of rise in surface water turbidity[18].

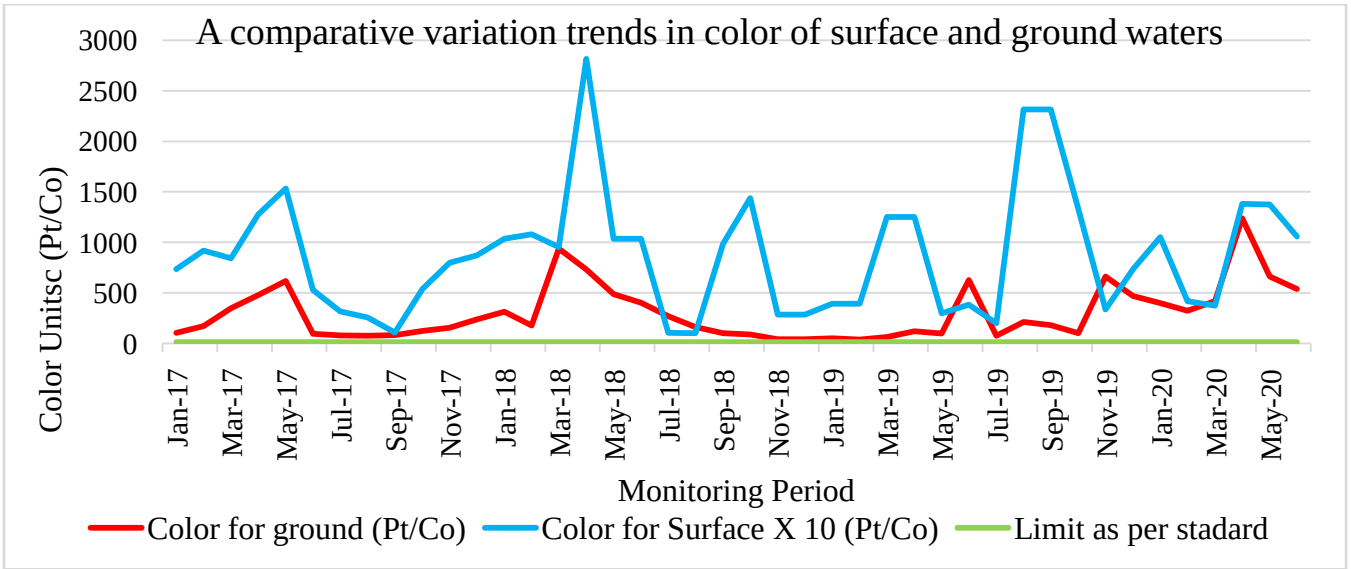


FIGURE 3: Plotted Color variations of surface and groundwater for Nyabarongo river and its groundwater aquifer respectively

As illustrated by Fig.3 the maximum values for color recorded during the whole monitoring period are in the rain seasons and a similar variation appear for groundwater because in all period of rise in color for Nyabarongo water groundwater turbidity has also risen. It is worth noting here that the color of groundwater can be caused by other parameters in water related to the geological nature of the area such as Manganese and Iron. However the variation in of color in groundwater proportionally to

the variation of river water is a proof that the quality of the river is surface water in the river has impact on groundwater quality [11].

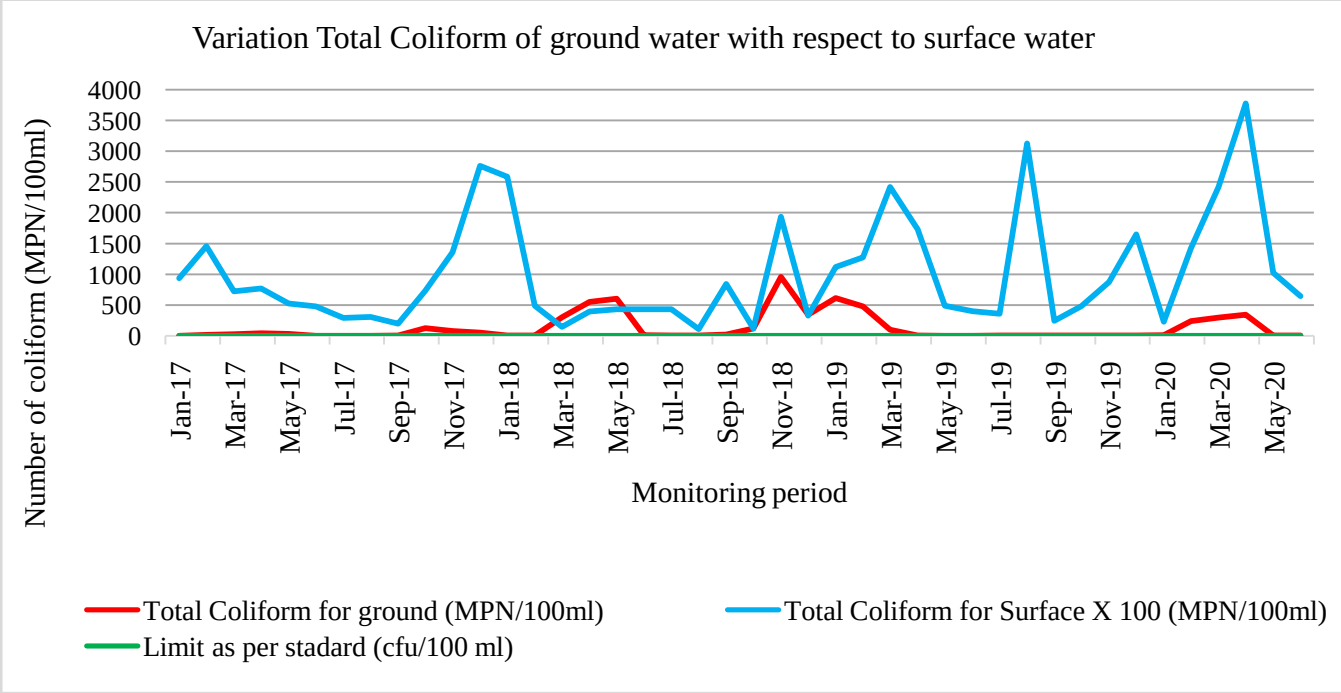


FIGURE 4: Plotted Total coliform variations of surface and groundwater for Nyabarongo river and its groundwater aquifer respectively

The literature indicates that the coliform bacteria in water are attached ot organic matter and other sediments loaded in water. In Nyabarongo river, the high values of total coliform during the rain season are related to the sediments and other contaminants loaded in the river during erosion and inundations[14]. The increase in value for the total coliform in ground water in the same periods a the variation for surface water indicate that the surface water quality has impact on groundwater.

IV. CONCLUSION

This study has used the results of laboratory analysis of turbidity, color and Total coliform parameters recorded in the period between January 2017 and June 2020 to assess the impact of the changes in Nyabarongo river quality as a result of flooding to the quality of groundwater. The findings indicated that turbidity and color highly rise most of the times in the rain seasons of March to May and October to December as a result of the sediments and other substances loaded in the river and its area during overflow and flooding [3]. The similar trend in water quality changes for both parameters on groundwater was observed which indicate that the pollution of surface water is impacting on groundwater provided the fact that the two water sources are hydrologically connected by the recharge [4]. The laboratory analyses were recorded on an hourly and daily basis which indicates the reliability of the results. However, the analysis did not cover the whole river length and the whole ground aquifer but the results of this study may represent the whole length based on the similarity in terms of physical and hydrological characteristics [1]. Another limitation is that the study did not analyze all parameters can be transferred through communication between surface and groundwater [9]. The findings of this study can serve as a reference for taking appropriate mitigation measure for protecting rivers in Rwanda from erosion and related pollution as well as for protection groundwater such as strategies for enhancing the natural treatment process of groundwater. This study can inform the institutions exploiting the river and its aquifer to incorporate technical measures to lessen the impacts on water quality such as to raise the wellhead at each borehole to a given height upward so that it is not submerged during flooding and installation of a groundwater telemetry system with for real time data capture and real time decision making.

ACKNOWLEDGEMENTS

The authors would like to express their thanks to the management Nzove Water Treatment Plant for the facilitations provided to conduct laboratory analyses and the provision of additional data. Authors are also grateful to all other data providers which contributed to the successful completion of this study.

REFERENCES

[1] WASAC (2020). Technical assessment and feasibility for the rehabilitation of Nzove I Water Treatment Plant and its supplying groundwaters. Final Report-Water and Sanitation Corporation Rwanda.

[2] Horner, R.R., Skupien, J.J., Livingston, E.H. and Shaver, H.E. (1994). Fundamentals of urban runoff management: technical and institutional issues. Terrene Institute, Washington, D.C.

[3] Weaver, J. C., Granato, G. E., & Fitzgerald, S. A. (2019). Assessing Water Quality from Highway Runoff at Selected Sites in North Carolina with the Stochastic Empirical Loading and Dilution Model (SELDM). Reston, VA: the North Carolina Department of Transportation, Division of Highways, Hydraulics Unit and the U.S. Department of Transportation, Federal Highway Administration, Office of Project Development and Environmental Review.

[4] Mosley, L., Sarabjeet S. and Aalbersberg, B. (2004): Water quality monitoring in Pacific Island countries. Handbook for water quality managers & laboratories, Public Health officers, water engineers and suppliers, Environmental Protection Agencies and all those organizations involved in water quality monitoring (1st Edition). 43 p; 30 cm, ISSN: 1605-4377: SOPAC, The University of the South Pacific. Suva - Fiji Islands.

[5] Rwanda Standards; RS EAC 12 (2018). Potable water - specifications. Third Edition. p.4-9. The Rwanda Standards Board.

[6] Jamie Bartram and Richard Balance (1996). Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes. United Nations Environment Programme and the World Health Organization © 1996 UNEP/WHO ISBN 0 419 22320 7 (Hbk) 0 419 21730 4 (Pbk).

[7] APHA 1992 Standard Methods for the Examination of Water and Wastewater. 18th edition, American Public Health Association (APHA), American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Washington, D.C. pp. 171–250.

[8] Z. Hoko, (2005); An Assessment of the Water Quality of Drinking Water in Rural Districts in Zimbabwe the Case of Gokwe South, Nkayi, Lupane, and Mwenezi Districts, Phys. Chem. Earth, 30, 859 – 866.

[9] Stelma GN, Jr, Wymer LJ. 2012. Research considerations for more effective groundwater monitoring. J. Water Health 10:511–521. 10.2166/wh.2012.016.

[10] MINITERE. (2006). National Adaptation Programmes of Action to climate change- NAPA final report. Kigali-Rwanda: Republic of Rwanda- Ministry of Lands, Environment, Forestry, Water and Mines-MINITERE.

[11] RWFA (2019). Water quality Monitoring. Phase I and II. Integrated water resources management in Rwanda, Final report. Rwanda Water and Forestry Authority, Kigali-Rwanda.

[12] RIWSP. (2016). Sediment Fingerprint for the Nyabarongo Upper Catchment in Rwanda-Final Technical report under USAID-Rwanda Cooperative Agreement No. AID-696-LA-11-00001USAID-Rwanda Cooperative Agreement No. AID-696-LA-11-00001 PHASEII. Florida-USA: Florida International University.

[13] RNRA (2017). Upper Nyabarongo catchment rehabilitation plan. Rwanda Natural Resources Authority; Final report Kigali-Rwanda.

[14] Ministry of Environment-Rwanda (2020). Erosion Control Mapping Report in Rwanda. MoE in partnership with the International Union for Conservation of Nature (IUCN) and the National Institute of Statistics of Rwanda (NISR).

[15] RWFA (2019). Ground Water Recharge and Storage Enhancement in Eastern Province. Rwanda IWRM programme.

[16] Ministry of Environment (2019). Upper Nyabarongo catchment management plan (2018-2024). MoE, Kigali-Rwanda.

[17] REMA. (2019). Rwanda Compendium of Environmental Statistics, First Edition-Final Report . Kigali-Rwanda: Rwanda Environment Management Authority.

[18] Nhapi I, U. W. (2011). Assessment of Water Pollution Levels in the Nyabugogo Catchment, Rwanda . Kigali-Rwanda: The Open Environmental Engineering journal, 2011, Vol. 4 , 40-53.

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