REPUBLIQUE DU CAMEROUN
Paix – Travail – Patrie
****
UNIVERSITE DE YAOUNDE I
FACULTE DES SCIENCES
DEPARTEMENT DE BIOCHIMIE
****
CENTRE DE RECHERCHE ET FORMATION DOCTORAL SCIENCE DE LA VIE, SANTE ET ENVIRONNEMENT SCIENCES (CRGS-LHES)
(CRFD-SVSE

REPUBLIC OF CAMEROUN
Peace – Work – Fatherland
****
UNIVERSITY OF YAOUNDE I
FACULTY OF SCIENCE
DEPARTMENT OF BIOCHEMISTRY
****
CENTRE FOR RESEARCH AND GRADUATE STUDIES IN LIFE, HEALTH & ENVIRONMENTAL

Evaluation of the seed performance of cocoyam (Xanthosoma sagittifolium L. Schott) minitubers under the influence of poultry manure and NPK fertilizer

THESIS
Submitted in partial fulfilment of the requirements for the award of a Doctorat/Ph.D Degree in Biochemistry

Par : **GWAN MOFOR ELVIS**
Master of Science Degree in Biochemistry

Sous la direction de
OMOKOLO NDOUMOU Denis
Professor

Année Académique : 2019-2020

REPUBLIQUE DU CAMEROUN
Paix—Travail – Patrie

UNIVERSITE DE YAOUNDE I

FACULTE DES SCIENCES
B.P. 812 Yaoundé

DEPARTEMENT DE BIOCHIMIE

REPUBLIC OF CAMEROON
Peace—Work – Fatherland

UNIVERSITY OF YAOUNDE I

FACULTY OF SCIENCE
P.O. Box 812 Yaounde

DEPARTMENT OF BIOCHEMISTRY

ATTESTATION DE CORRECTION DE THESE DE DOCTORAT/Ph.D

Nous soussignés, **Professeur OBEN ENYONG Julius**, Président et Membres du jury de Soutenance de la Thèse de Doctorat/Ph.D de **Monsieur GWAN MOFOR Elvis**, étudiant au département de Biochimie, **Matricule 09R1309**, Né le 06 Novembre 1979 à Bali, autorisée par la correspondance **N° 20-2938/UYI/VREPDTIC/DAAC/DEPE/SPD/CB-AP** de Monsieur le Recteur de l'Université de Yaoundé 1 en date du 02 Decembre 2020.

Attestons qu'après la soutenance publique de sa Thèse de Doctorat/Ph.D en date du 21 Decembre 2020 à l'Université de Yaoundé 1, il a effectué toutes les corrections conformément aux remarques et suggestions des membres du Jury.

En foi de quoi la présente attestation lui est délivrée pour servir et valoir ce que de droit.

Yaoundé le 03 FEV 2021

Président du Jury

Julius E. Oben P.h.d.
Professor

Membres du Jury

Pr. BOUDJEKO Thaddée, Ph. D
Maître de Conférences
Université de Yaoundé 1

FORMAL LIST OF PERMANENT TEACHING STAFF OF THE FACULTY OF SCIENCE

UNIVERSITÉ DE YAOUNDÉ I **Faculté des Sciences** Division de la Programmation et du Suivi des Activités Académiques		THE UNIVERSITY OF YAOUNDE I **Faculty of Science** Division of Programming and Follow-up of Academic Affaires
LISTE DES ENSEIGNANTS PERMANENTS		**LIST OF PERMANENT TEACHING STAFF**

ANNÉE ACADEMIQUE 2019/2020

(Par Département et par Grade)

DATE D'ACTUALISATION 19 Février 2020

ADMINISTRATION

- **DOYEN :** TCHOUANKEU Jean- Claude, *Maitre de Conférences*
- **VICE-DOYEN / DPSAA :** DONGO Etienne, *Professeur*
- **VICE-DOYEN / DSSE :** AJEAGAH Gideon AGHAINDUM, *Professeur*
- **VICE-DOYEN / DRC :** ABOSSOLO Monique, *Maitre de Conférences*
- **Chef Division Administrative et Financière :** NDOYE FOE Marie C. F., *Maitre de Conférences*
- **Chef Division des Affaires Académiques, de la Scolarité et de la Recherche DAASR :** MBAZE MEVA'A Luc Léonard, *Professeur*

1- DÉPARTEMENT DE BIOCHIMIE (BC) (38)

N°	NOMS ET PRÉNOMS	GRADE	OBSERVATIONS
1	BIGOGA DIAGA Jude	Professeur	En poste
2	FEKAM BOYOM Fabrice	Professeur	En poste
3	FOKOU Elie	Professeur	En poste
4	KANSCI Germain	Professeur	En poste
5	MBACHAM FON Wilfried	Professeur	En poste
6	MOUNDIPA FEWOU Paul	Professeur	Chef de Département
7	NINTCHOM PENLAP V. épse BENG	Professeur	En poste
8	OBEN Julius ENYONG	Professeur	En poste
9	ACHU Merci BIH	Maître de Conférences	En poste
10	ATOGHO Barbara Mma	Maître de Conférences	En poste
11	AZANTSA KINGUE GABIN BORIS	Maître de Conférences	En poste
12	BELINGA née NDOYE FOE M. C. F.	Maître de Conférences	Chef DAF / FS
13	BOUDJEKO Thaddée	Maître de Conférences	En poste
14	DJUIDJE NGOUNOUE Marcelline	Maître de Conférences	En poste
15	EFFA NNOMO Pierre	Maître de Conférences	En poste
16	NANA Louise épouse WAKAM	Maître de Conférences	En poste
17	NGONDI Judith Laure	Maître de Conférences	En poste
18	NGUEFACK Julienne	Maître de Conférences	En poste
19	NJAYOU Frédéric Nico	Maître de Conférences	En poste
20	MOFOR née TEUGWA Clotilde	Maître de Conférences	Inspecteur de Service MINESUP
21	TCHANA KOUATCHOUA Angèle	Maître de Conférences	En poste

22	AKINDEH MBUH NJI	Chargée de Cours	En poste
23	BEBOY EDZENGUELE Sara Nathalie	Chargée de Cours	En poste
24	DAKOLE DABOY Charles	Chargée de Cours	En poste
25	DJUIKWO NKONGA Ruth Viviane	Chargée de Cours	En poste
26	DONGMO LEKAGNE Joseph Blaise	Chargé de Cours	En poste
27	EWANE Cécile Anne	Chargée de Cours	En poste
28	FONKOUA Martin	Chargé de Cours	En poste
29	BEBEE Fadimatou	Chargée de Cours	En poste
30	KOTUE KAPTUE Charles	Chargé de Cours	En poste
31	LUNGA Paul KEILAH	Chargé de Cours	En poste
32	MANANGA Marlyse Joséphine	Chargée de Cours	En poste
33	MBONG ANGIE M. Mary Anne	Chargée de Cours	En poste
34	PECHANGOU NSANGOU Sylvain	Chargé de Cours	En poste
35	Palmer MASUMBE NETONGO	Chargé de Cours	En poste
36	MBOUCHE FANMOE Marceline Joëlle	Assistante	En poste
37	OWONA AYISSI Vincent Brice	Assistant	En poste
38	WILFRIED ANGIE Abia	Assistant	En poste

2- DÉPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE ANIMALES (BPA) (48)			
1	AJEAGAH Gideon AGHAINDUM	Professeur	*VICE-DOYEN / DSSE*
2	BILONG BILONG Charles-Félix	Professeur	Chef de Département
3	DIMO Théophile	Professeur	En Poste
4	DJIETO LORDON Champlain	Professeur	En Poste
5	ESSOMBA née NTSAMA MBALA	Professeur	*Vice Doyen/FMSB/UYI*
6	FOMENA Abraham	Professeur	En Poste
7	KAMTCHOUING Pierre	Professeur	En poste
8	NJAMEN Dieudonné	Professeur	En poste
9	NJIOKOU Flobert	Professeur	En Poste
10	NOLA Moïse	Professeur	En poste
11	TAN Paul VERNYUY	Professeur	En poste
12	TCHUEM TCHUENTE Louis Albert	Professeur	*Inspecteur de service* *Coord.Progr./MINSANTE*
13	ZEBAZE TOGOUET Serge Hubert	Professeur	*En poste*
14	BILANDA Danielle Claude	Maître de Conférences	En poste
15	DJIOGUE Séfirin	Maître de Conférences	En poste
16	DZEUFIET DJOMENI Paul Désiré	Maître de Conférences	En poste
17	JATSA BOUKENG Hermine épse MEGAPTCHE	Maître de Conférences	En Poste
18	KEKEUNOU Sévilor	Maître de Conférences	En poste
19	MEGNEKOU Rosette	Maître de Conférences	En poste
20	MONY Ruth épse NTONE	Maître de Conférences	En Poste
21	NGUEGUIM TSOFACK Florence	Maître de Conférences	En poste
22	TOMBI Jeannette	Maître de Conférences	En poste
23	ALENE Désirée Chantal	Chargée de Cours	En poste
26	ATSAMO Albert Donatien	Chargé de Cours	En poste
27	BELLET EDIMO Oscar Roger	Chargé de Cours	En poste
28	DONFACK Mireille	Chargée de Cours	En poste
29	ETEME ENAMA Serge	Chargé de Cours	En poste
30	GOUNOUE KAMKUMO Raceline	Chargée de Cours	En poste
31	KANDEDA KAVAYE Antoine	Chargé de Cours	En poste
32	LEKEUFACK FOLEFACK Guy B.	Chargé de Cours	En poste
33	MAHOB Raymond Joseph	Chargé de Cours	En poste
34	MBENOUN MASSE Paul Serge	Chargé de Cours	En poste

35	MOUNGANG LucianeMarlyse	Chargée de Cours	En poste
36	MVEYO NDANKEU Yves Patrick	Chargé de Cours	En poste
37	NGOUATEU KENFACK Omer Bébé	Chargé de Cours	En poste
38	NGUEMBOK	Chargé de Cours	En poste
39	NJUA Clarisse Yafi	Chargée de Cours	Chef Div. UBA
40	NOAH EWOTI Olive Vivien	Chargée de Cours	En poste
41	TADU Zephyrin	Chargé de Cours	En poste
42	TAMSA ARFAO Antoine	Chargé de Cours	En poste
43	YEDE	Chargé de Cours	En poste
44	BASSOCK BAYIHA Etienne Didier	Assistant	En poste
45	ESSAMA MBIDA Désirée Sandrine	Assistante	En poste
46	KOGA MANG DOBARA	Assistant	En poste
47	LEME BANOCK Lucie	Assistante	En poste
48	YOUNOUSSA LAME	Assistant	En poste

3- DÉPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE VÉGÉTALES (BPV) (32)			
1	AMBANG Zachée	Professeur	Chef Division/UYII
2	BELL Joseph Martin	Professeur	En poste
3	DJOCGOUE Pierre François	Professeur	En poste
4	MOSSEBO Dominique Claude	Professeur	En poste
5	YOUMBI Emmanuel	Professeur	Chef de Département
6	ZAPFACK Louis	Professeur	En poste
7	ANGONI Hyacinthe	Maître de Conférences	En poste
8	BIYE Elvire Hortense	Maître de Conférences	En poste
9	KENGNE NOUMSI Ives Magloire	Maître de Conférences	En poste
10	MALA Armand William	Maître de Conférences	En poste
11	MBARGA BINDZI Marie Alain	Maître de Conférences	CT/ MINESUP
12	MBOLO Marie	Maître de Conférences	En poste
13	NDONGO BEKOLO	Maître de Conférences	*CE / MINRESI*
14	NGONKEU MAGAPTCHE Eddy L.	Maître de Conférences	En poste
15	TSOATA Esaïe	Maître de Conférences	En poste
16	TONFACK Libert Brice	Maître de Conférences	En poste
17	DJEUANI Astride Carole	Chargé de Cours	En poste
18	GOMANDJE Christelle	Chargée de Cours	En poste
19	MAFFO MAFFO Nicole Liliane	Chargé de Cours	En poste
20	MAHBOU SOMO TOUKAM. Gabriel	Chargé de Cours	En poste
21	NGALLE Hermine BILLE	Chargée de Cours	En poste
22	NGOUO Lucas Vincent	Chargé de Cours	En poste
23	NNANGA MEBENGA Ruth Laure	Chargé de Cours	En poste
24	NOUKEU KOUAKAM Armelle	Chargé de Cours	En poste
25	ONANA JEAN MICHEL	Chargé de Cours	En poste
26	GODSWILL NTSOMBAH NTSEFONG	Assistant	En poste
27	KABELONG BANAHO Louis-Paul-Roger	Assistant	En poste
28	KONO Léon Dieudonné	Assistant	En poste
29	LIBALAH Moses BAKONCK	Assistant	En poste
30	LIKENG-LI-NGUE Benoit C	Assistant	En poste
31	TAEDOUNG Evariste Hermann	Assistant	En poste
32	TEMEGNE NONO Carine	Assistant	En poste

4- DÉPARTEMENT DE CHIMIE INORGANIQUE (CI) (35)			
1	AGWARA ONDOH Moïse	Professeur	*Vice RecteurUniv ,Bamenda*
2	ELIMBI Antoine	Professeur	En poste
3	Florence UFI CHINJE épouse MELO	Professeur	*Recteur Univ.Ngaoundere*
4	GHOGOMU Paul MINGO	Professeur	*Ministre Chargé deMiss.PR*
5	NANSEU Njiki Charles Péguy	Professeur	En poste
6	NDIFON Peter TEKE	Professeur	*CT MINRESI/Chef de Departement*
7	NGOMO Horace MANGA	Professeur	*Vice Chancelor/UB*
7	NDIKONTAR Maurice KOR	Professeur	*Vice-Doyen Univ. Bamenda*
8	NENWA Justin	Professeur	En poste
9	NGAMENI Emmanuel	Professeur	*DOYEN FS UDs*
10	BABALE née DJAM DOUDOU	Maître de Conférences	*Chargée Mission P.R.*
11	DJOUFAC WOUMFO Emmanuel	Maître de Conférences	En poste
12	KAMGANG YOUBI Georges	Maître de Conférences	En poste
13	KEMMEGNE MBOUGUEM Jean C.	Maître de Conférences	En poste
14	KONG SAKEO	Maître de Conférences	En poste
15	NDI NSAMI Julius	Maître de Conférences	En poste
17	NJIOMOU C. épse DJANGANG	Maître de Conférences	En poste
18	NJOYA Dayirou	Maître de Conférences	En poste
19	YOUNANG Elie	Maître de Conférences	En poste
20	ACAYANKA Elie	Chargé de Cours	En poste
21	BELIBI BELIBI Placide Désiré	Chargé de Cours	CS/ ENS Bertoua
22	CHEUMANI YONA Arnaud M.	Chargé de Cours	En poste
23	EMADACK Alphonse	Chargé de Cours	En poste
24	KENNE DEDZO GUSTAVE	Chargé de Cours	En poste
25	KOUOTOU DAOUDA	Chargé de Cours	En poste
26	MAKON Thomas Beauregard	Chargé de Cours	En poste
27	MBEY Jean Aime	Chargé de Cours	En poste
28	NCHIMI NONO KATIA	Chargé de Cours	En poste
29	NEBA nee NDOSIRI Bridget NDOYE	Chargée de Cours	CT/ MINFEM
30	NYAMEN Linda Dyorisse	Chargée de Cours	En poste
31	PABOUDAM GBAMBIE A.	Chargée de Cours	En poste
32	TCHAKOUTE KOUAMO Hervé	Chargé de Cours	En poste
33	NJANKWA NJABONG N. Eric	Assistant	En poste
34	PATOUOSSA ISSOFA	Assistant	En poste
35	SIEWE Jean Mermoz	Assistant	En Poste

5- DÉPARTEMENT DE CHIMIE ORGANIQUE (CO) (35)			
1	DONGO Etienne	Professeur	Vice-Doyen / PSAA
2	GHOGOMU TIH Robert Ralph	Professeur	Dir. IBAF/UDA
3	NGOUELA Silvère Augustin	Professeur	Chef de Departement UDS
4	NKENGFACK Augustin Ephrem	Professeur	Chef de Département
5	NYASSE Barthélemy	Professeur	En poste
6	PEGNYEMB Dieudonné Emmanuel	Professeur	*Directeur/ MINESUP*
7	WANDJI Jean	Professeur	En poste
8	Alex de Théodore ATCHADE	Maître de Conférences	*DEPE/ Rectorat/UYI*
9	EYONG Kenneth OBEN	Maître de Conférences	*Chef Service Programme & Diplomes*
10	FOLEFOC Gabriel NGOSONG	Maître de Conférences	En poste
11	FOTSO WABO Ghislain	Maître de Conférences	En poste
12	KEUMEDJIO Félix	Maître de Conférences	En poste

13	KEUMOGNE Marguerite	Maître de Conférences	En poste
14	KOUAM Jacques	Maître de Conférences	En poste
15	MBAZOA née DJAMA Céline	Maître de Conférences	En poste
16	MKOUNGA Pierre	Maître de Conférences	En poste
17	NOTE LOUGBOT Olivier Placide	Maître de Conférences	Chef Service/MINESUP
18	NGO MBING Joséphine	Maître de Conférences	Sous/Direct. MINERESI
19	NGONO BIKOBO Dominique Serge	Maître de Conférences	En poste
20	NOUNGOUE TCHAMO Diderot	Maître de Conférences	En poste
21	TABOPDA KUATE Turibio	Maître de Conférences	En poste
22	TCHOUANKEU Jean-Claude	Maître de Conférences	*Doyen /FS/ UYI*
23	TIH née NGO BILONG E. Anastasie	Maître de Conférences	En poste
24	YANKEP Emmanuel	Maître de Conférences	En poste
25	AMBASSA Pantaléon	Chargé de Cours	En poste
26	KAMTO Eutrophe Le Doux	Chargé de Cours	En poste
27	MVOT AKAK CARINE	Chargé de Cours	En poste
28	NGNINTEDO Dominique	Chargé de Cours	En poste
29	NGOMO Orléans	Chargée de Cours	En poste
30	OUAHOUO WACHE Blandine M.	Chargée de Cours	En poste
31	SIELINOU TEDJON Valérie	Chargé de Cours	En poste
32	TAGATSING FOTSING Maurice	Chargé de Cours	En poste
33	ZONDENDEGOUMBA Ernestine	Chargée de Cours	En poste
34	MESSI Angélique Nicolas	Assistant	En poste
35	TSEMEUGNE Joseph	Assistant	En poste

6- DÉPARTEMENT D'INFORMATIQUE (IN) (27)			
1	ATSA ETOUNDI Roger	Professeur	*Chef Div.MINESUP*
2	FOUDA NDJODO Marcel Laurent	Professeur	*Chef Dpt ENS/Chef IGA.MINESUP*
3	NDOUNDAM Réné	Maître de Conférences	En poste
4	AMINOU Halidou	Chargé de Cours	En poste
5	DJAM Xaviera YOUH - KIMBI	Chargé de Cours	En Poste
6	EBELE Serge Alain	Chargé de Cours	En poste
7	KOUOKAM KOUOKAM E. A.	Chargé de Cours	En poste
8	MELATAGIA YONTA Paulin	Chargé de Cours	En poste
9	MOTO MPONG Serge Alain	Chargé de Cours	En poste
10	TAPAMO Hyppolite	Chargé de Cours	En poste
11	ABESSOLO ALO'O Gislain	Chargé de Cours	En poste
12	KAMGUEU Patrick Olivier	Chargé de Cours	En poste
13	MONTHE DJIADEU Valery M.	Chargé de Cours	En poste
14	OLLE OLLE Daniel Claude Delort	Chargé de Cours	C/D Enset. Ebolowa
15	TINDO Gilbert	Chargé de Cours	En poste
16	TSOPZE Norbert	Chargé de Cours	En poste
17	WAKU KOUAMOU Jules	Chargé de Cours	En poste
18	BAYEM Jacques Narcisse	Assistant	En poste
19	DOMGA KOMGUEM Rodrigue	Assistant	En poste
20	EKODECK Stéphane Gaël Raymond	Assistant	En poste
21	HAMZA Adamou	Assistant	En poste
22	JIOMEKONG AZANZI Fidel	Assistant	En poste
23	MAKEMBE. S . Oswald	Assistant	En poste
24	MESSI NGUELE Thomas	Assistant	En poste
25	MEYEMDOU Nadège Sylvianne	Assistante	En poste
26	NKONDOCK. MI. BAHANACK.N.	Assistant	En poste

7- DÉPARTEMENT DE MATHÉMATIQUES (MA) (30)			
1	EMVUDU WONO Yves S.	Professeur	*CD Info/ Inspecteur MINESUP*
2	AYISSI Raoult Domingo	Maître de Conférences	Chef de Département
3	NKUIMI JUGNIA Célestin	Maître de Conférences	En poste
4	NOUNDJEU Pierre	Maître de Conférences	En poste
5	MBEHOU Mohamed	Maître de Conférences	En poste
6	TCHAPNDA NJABO Sophonie B.	Maître de Conférences	Directeur/AIMS Rwanda
7	AGHOUKENG JIOFACK Jean Gérard	Chargé de Cours	Chef Cellule MINPLAMAT
8	CHENDJOU Gilbert	Chargé de Cours	En poste
9	DJIADEU NGAHA Michel	Chargé de Cours	En poste
10	DOUANLA YONTA Herman	Chargé de Cours	En poste
11	FOMEKONG Christophe	Chargé de Cours	En poste
12	KIANPI Maurice	Chargé de Cours	En poste
13	KIKI Maxime Armand	Chargé de Cours	En poste
14	MBAKOP Guy Merlin	Chargé de Cours	En poste
15	MBANG Joseph	Chargé de Cours	En poste
16	MBELE BIDIMA Martin Ledoux	Chargé de Cours	En poste
17	MENGUE MENGUE David Joe	Chargé de Cours	En poste
18	NGUEFACK Bernard	Chargé de Cours	En poste
19	NIMPA PEFOUNKEU Romain	Chargée de Cours	En poste
20	POLA DOUNDOU Emmanuel	Chargé de Cours	En poste
21	TAKAM SOH Patrice	Chargé de Cours	En poste
22	TCHANGANG Roger Duclos	Chargé de Cours	En poste
23	TCHOUNDJA Edgar Landry	Chargé de Cours	En poste
24	TETSADJIO TCHILEPECK M. E.	Chargée de Cours	En poste
25	TIAYA TSAGUE N. Anne-Marie	Chargée de Cours	En poste
26	MBIAKOP Hilaire George	Assistant	En poste
27	BITYE MVONDO Esther Claudine	Assistante	En poste
28	MBATAKOU Salomon Joseph	Assistant	En poste
29	MEFENZA NOUNTU Thiery	Assistant	En poste
30	TCHEUTIA Daniel Duviol	Assistant	En poste

8- DÉPARTEMENT DE MICROBIOLOGIE (MIB) (18)			
1	ESSIA NGANG Jean Justin	Professeur	DRV/IMPM
2	BOYOMO ONANA	Maître de Conférences	En poste
3	NWAGA Dieudonné M.	Maître de Conférences	En poste
4	NYEGUE Maximilienne Ascension	Maître de Conférences	En poste
5	RIWOM Sara Honorine	Maître de Conférences	En poste
6	SADO KAMDEM Sylvain Leroy	Maître de Conférences	En poste
7	ASSAM ASSAM Jean Paul	Chargé de Cours	En poste
8	BODA Maurice	Chargé de Cours	En poste
9	BOUGNOM Blaise Pascal	Chargé de Cours	En poste
10	ESSONO OBOUGOU Germain G.	Chargé de Cours	En poste
11	NJIKI BIKOÏ Jacky	Chargée de Cours	En poste
12	TCHIKOUA Roger	Chargé de Cours	En poste
13	ESSONO Damien Marie	Assistante	En poste
14	LAMYE Glory MOH	Assistant	En poste
15	MEYIN A EBONG Solange	Assistante	En poste
16	NKOUDOU ZE Nardis	Assistant	En poste
17	SAKE NGANE Carole Stéphanie	Assistante	En poste
18	TOBOLBAÏ Richard	Assistant	En poste

9. DEPARTEMENT DE PYSIQUE(PHY) (42)			
1	BEN- BOLIE Germain Hubert	Professeur	En poste
2	ESSIMBI ZOBO Bernard	Professeur	En poste
3	KOFANE Timoléon Crépin	Professeur	En poste
4	NANA ENGO Serge Guy	Professeur	En poste
5	NDJAKA Jean Marie Bienvenu	Professeur	Chef de Département
6	NOUAYOU Robert	Professeur	En poste
7	NJANDJOCK NOUCK Philippe	Professeur	*Sous Directeur/ MINRESI*
8	PEMHA Elkana	Professeur	En poste
9	TABOD Charles TABOD	Professeur	Doyen Univ/Bda
10	TCHAWOUA Clément	Professeur	En poste
11	WOAFO Paul	Professeur	En poste
12	BIYA MOTTO Frédéric	Maître de Conférences	DG/HYDRO Mekin
13	BODO Bertrand	Maître de Conférences	En poste
14	DJUIDJE KENMOE épouse ALOYEM	Maître de Conférences	En poste
15	EKOBENA FOUDA Henri Paul	Maître de Conférences	*Chef Division. UN*
16	EYEBE FOUDA Jean sire	Maître de Conférences	En poste
17	FEWO Serge Ibraïd	Maître de Conférences	En poste
18	HONA Jacques	Maître de Conférences	En poste
19	MBANE BIOUELE César	Maître de Conférences	En poste
20	NANA NBENDJO Blaise	Maître de Conférences	En poste
21	NDOP Joseph	Maître de Conférences	En poste
22	SAIDOU	Maître de Conférences	MINERESI
23	SIEWE SIEWE Martin	Maître de Conférences	En poste
24	SIMO Elie	Maître de Conférences	En poste
25	VONDOU Derbetini Appolinaire	Maître de Conférences	En poste
26	WAKATA née BEYA Annie	Maître de Conférences	*Sous Directeur/ MINESUP*
27	ZEKENG Serge Sylvain	Maître de Conférences	En poste
28	ABDOURAHIMI	Chargé de Cours	En poste
29	EDONGUE HERVAIS	Chargé de Cours	En poste
30	ENYEGUE A NYAM épse BELINGA	Chargée de Cours	En poste
31	FOUEDJIO David	Chargé de Cours	Chef Cell. MINADER
32	MBINACK Clément	Chargé de Cours	En poste
33	MBONO SAMBA Yves Christian U.	Chargé de Cours	En poste
34	MELI'I Joelle Larissa	Chargée de Cours	En poste
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DEDICATION

To:

- My Late parents, **GWAN Aaron CHOH** and **GWAN Agie LUM**

- My lovely wife **GWAN MOFOR Sharon** and wonderful children, **Lynn-Blessing LUM, Hans- Aaron CHOH** and **David- Ken MUMA** for the conducive home environment provided for this work to be carried out

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LIST OF ABBREVIATIONS

B	:Blank
C	:Control
CEC	:Cation exchange capacity
CV	:Cultivar
FW	:Fresh weight
FAs	:Fulvic adds
FAO	:Food and agricultural Organization
HAs	:Humic adds
HPLC	High performance liquid chromatography
MAP	:Months after planting
MS	:Mass spectrometry
NPK	:Nitrogen, Phosphorus, Potassium
PM	:Poultry manure
POM	:Particulate organic matter
PPO	:Polyphenol oxidase
ROS	:Reactive oxygen species
SPSS	:Statistical Package for Social Sciences
SD	:Short day
UV	:Ultra violet
WAP	:Weeks after planting

ABSTRACT

The response of white and red cultivar (cv) cocoyam *(Xanthosoma sagittifolium* (L.) Schott) minitubers used as seeds was evaluated in different treatments. Poultry manure (PM) and NPK (20:10:10) fertilizer were applied. The experimental farm was situated at Ngog Bibega, Mbankomo Sub-division, Mefou and Afamba division, Centre region, Cameroon. Treatment combinations comprising of three rates each of PM (0, 20 and 30t.ha^{-1}) and NPK (0, 120 and 150 kg.ha^{-1}) were factorized and fitted into a randomized complete block design with three replicates. Physico-chemical analysis of all treatment plots revealed clay loam textures. Poultry manure treatment plots significantly ($P<0.05$) increased soil pH, organic content, total carbon and cation exchange capacity. At 6 months of culture of both cultivars, the agronomic parameters evaluated (average plant height, average leaf number and average leaf area) under poultry manure (30t.ha^{-1}) treatments were higher compared to those of other treatments. Under the same treatments of poultry manure (30t.ha^{-1}), the white cv plants recorded the highest mean yield parameters 9 months after planting among all treatments. For white cv plants, the average tuber number per plant was 8, average tuber weight was 250g, average tuber length was 14cm and average tuber girth was 18.5cm compared to the red cultivar average yield parameters; average tuber number per plant (5.7), average tuber weight (124.7g), average tuber length (8cm) and average tuber girth (14.5cm). The nutritional analysis of harvested tubers showed that red cv *X. sagittifolium* tubers (cormels) had a higher crude protein content than the white cv *X. sagittifolium* tubers for all treatments. Results obtained from the analysis of the macromineral content of harvested tubers showed that calcium, magnesium, sodium, phosphorus and nitrogen were higher in the red cv tubers than in the white cv tubers for all treatments. Biochemical analyses showed that the treatments tested, significantly ($P<0.05$) influenced the content of secondary metabolites evaluated in the leaves. Oxidase activities (peroxidases, polyphenol oxidase and glucanase) were evaluated in the different treatments. After 2 months of culture, plants treated with NPK (150kg.ha^{-1}) expressed highest phenolic contents (1.22±0.13mg eq catechin. g^{-1} FW) and flavonoid contents (1.08±0.16mg g^{-1} FW) in white cv. After 6 months of culture, white cv plants treated with the poultry manure (30t.ha^{-1}) epressed the highest protein content (5.04±0.38mg eq BSA.g^{-1} FW) and the most significant ($P<0.05$) peroxidase activity (4.89±1.36 UE min^{-1}). On the contrary when white cv plants were treated with NPK (150kg.ha^{-1}) fertilizer they recorded the most significant ($P<0.05$) polyphenol oxidase activity (4.99±0.10 in D330 nm^{-1}. min^{-1} UE^{-1}. g^{-1} FW) after 6 months of culture. In the red cv plants treated with NPK (150kg.ha^{-1})

fertilizer, glucanase activity the was most significant ($P<0.05$) (9.33±1.17 mg eq glucose g^{-1} FW) after 2 months of culture. HPLC-DAD-MS analyses permitted the identification of 8 flavonoid compounds in the leaf extracts of white and red cv plants under different fertilizer treatments. In all treatments our results illustrated two newly identified flavone C- glycosides (2 isomers of apeginin) and six known flavone C-glycosides (6, 8-Di-C-glucopyranosylapigenin, Isovitexin 6"-O-glucopyranoside, Apigenin 6-C-glucoside 8-C-arabinoside, vitexin, isovitexin and. 2"-O-Malonylvitexin). After 6 months of growth, poultry manure treatments (30t.ha^{-1}) enhanced flavone production in both cultivars compared to NPK fertilizer and control treatments. These results also showed: positive and significant correlations between growth and yield parameters, positive and non significant correlations between yield and biochemical parameters in both cultivars. Results obtained from this pioneering field trial of *Xanthosoma sagittifolium* minitubers, indicate that under certain conditions, these minitubers can serve as seeds to solve health problems linked to the use of tuber fragments as planting material.

Key words: poultry manure, NPK fertilizer, flavone c- glycosides, *Xanthosoma sagittifolium,* chemotaxonomic marker, macrominerals.

RESUME

Le comportement des mintubercules de macabo (*Xanthosoma sagittifolium.* cultivar (cv) blanc et rouge) utilisés comme semence, a été evalué dans differents traitements. La fiente de poules et l'engrais NPK (20:10:10) ont été appliqués. Le champ expérimental se situe à Ngog Bibiga, dans l'arrondissement de Mbankomo, departement de la Mefou et Afamba, region du Centre, Cameroun. Pour la fiente de poules les traitements 0t/ha, 20t/ha et 30t/ha ont été testés. Pour l'engrais NPK les traitements 0kg/ha, 120kg/ha et 150kg/ha ont également été testés. Les traitements ont été réalisés dans un modèle de bloc complet randomisé avec trois répétitions. L'analyse physico-chimique de toutes les parcelles ayant reçu le traitement a révélé une texture du sol limoneux-argileux. Dans les sols ayant reçu les traitements de la fiente de poules on note une augmentation significative ($P < 0,05$), du pH du sol, de la teneur en matières organiques, du carbone total et de la capacité d'échange cationique. Chez les 2 cvs et après 6 mois de culture les paramètres agronomiques évalués (taille moyen des plantes, nombre moyen de feuilles et la surface foliaire moyenne) sont plus élevés chez les plantes traiteés avec 30t/ha de fiente de poules par rapport aux autres traitements. De même le cv blanc donne un rendement plus élevés que le cv rouge après 9 mois de culture pour tous les traitements. A ce stade et toujours chez le cv blanc pour le traitement fiente de poules (30t/ha); le nombre moyen de tubercule par plante est de 8, le poids moyen du tubercule est de 250g, la longueur moyenne du tubercule est de 14cm et la circonference moyenne du tubercule est de 18,5cm. Dans les mêmes conditions chez le cv rouge le nombre moyen de tubercule par plante est de 5,7, le poids moyen du tubercule est de 124,7g, la longueur moyenne du tubercule est de 8cm et la circonference moyenne du tubercule est de 14,5cm. L'analyse des proprietés nutritionelles des tubercules a montré que le cv rouge a une teneur en protéines brutes plus éleveés que les tubercules du cv blanc dans tous les traitements. L'évaluation de la teneur en macroéléments des tubercules a montré que les teneurs en calcium, magnésium, sodium, phosphore et l'azote sont plus éleveés chez le cv rouge par rapport au cv blanc et dans tous les traitements. Les analyses biochimiques ont montré que les traitements testés influencent significativement ($P < 0,05$) les teneurs en métabolites secondaires évalués dans les feuilles. Les activités oxydases (peroxydases, polyphenol oxydases et glucanases) ont été evalués dans les differents traitements. Après 2 mois de culture les plantes traiteés au NPK (150kg/ha) ont des teneurs les plus éleveés en composées phénoliques ($1,22 \pm 0,13$ mg eq de catéchine/g MF) et en flavonoïdes ($1,08 \pm 0,16$ mg /g MF) chez le cv blanc. Après 6 mois de culture les plantes du cv blanc, traiteés a la fiente de poules

(30t/ha) ont des teneurs en protéines la plus éleveés (5,04 ± 0,38 mg éq BSA/g MF) et une activité peroxydase totale plus forte (4,89 ± 1,36 UE /min). Par contre lorsque le cv blanc est traité au NPK à 150kg/ha et après 6 mois de culture c'est l'activité polyphénol oxydases qui est la plus éleveés (4,99 ± 0,10 dans D330 /nm/Min/ UE/g MF). Chez le cv rouge traité au NPK à 150kg/ha c'est plutot l'activité glucanase qui est la plus eleveés (9,33 ± 1,17 mg eq glucose/ g MF) après 2 mois de culture. Les analyses effectuent avec le HPLC-DAD-MS ont permis d'identifier huit composés flavonoïdes dans les extraits de feuilles des cultivars blanc et rouge dans differents traitements. Nos analyses ont permis d'identifier deux nouveau glycosides C-flavone (2 isomères de l'apéginine) et six glycosides C- flavones connus (6,8-Di-C-glucopyranosylapigénine, Isovitexine 6 " - O-glucopyranoside, Apigénine 6-C-glucoside 8 - C-arabinoside, vitexine, isovitexine et 2 "- O-malonylvitexine). Après 6 mois de culture les traitements à la fiente de poules (30t/ha) augmentent la teneur des flavones chez le 2 cultivars par rapport au NPK et aux témoins. Nos resultats ont montrés; les correlations positives et significative entre les paramètres de croissance et les paramètres de rendement; les correlations positives et non significative entre les paramètres de rendement et le paramètres biochimiques dans les deux cultivars. Les résultats obtenus indiquent que les mintubercules de *Xanthosoma sagittifolium* peuvent étre utilisé dans certaines conditions comme semences pour resourdre les problèmes sanitaires lies à l'utilisation des fragments de tubercules comme matériel de plantation.

Mots clés: fiente de poules, engrais NPK, HPLC-DAD-MS, glycosides C- flavones, *X. sagittifolium*, marqueur chimio taxonomique, macroéléments.

INTRODUCTION

Cocoyam (*Xanthosoma sagittifolium* L. Schott, Araceae) is a herbaceous plant cultivated in tropical and subtropical regions for its edible tubers and leaves. Cocoyam tubers and leaves are essentially rich in sugars, proteins, vitamins and mineral salts (Sefa-Dedeh and Agyir-Sackey 2004). It covers the food needs of more than 200 million persons in the tropical and subtropical regions and more than 400 million persons worldwide (Onopkise *et al.,* 1999). It occupies the sixth position worldwide with an annual production of 0.45 million metric tonnes (Anonymous, 2006) and the second in Cameroon after cassava (*Mannihot esculenta*) in terms of tuber production.

In Cameroon, the unavailability of healthy seeds to farmers has affected the national production rate. The infestation of seeds is due to several viral, fungal and bacterial diseases (Anonymous, 2008; Chen and Adams, 2001). Otherwise, the lack of *X. sagittifolium* seeds can be justified by the fact that the plant usually propagates vegetatively from tuber fragments. This increases the dissemination of many pathogens which cause rot disease in cocoyam such as *Pythium myriotylum* (Boudjeko *et al.,* 2005), *Fusarium oxysporum* and *Fusarium solani* (Ubalua and Chukwu, 2008; Anele and Nwawuisi, 2008) and Dasheen mosaic virus that is found in the leaves, corms and cormels (Chen and Adams 2001). To overcome such problems, efforts have been made to improve the application of tissue culture technology to *X. sagittifolium* production through *in vitro* regeneration of *X. sagittifolium* plants using biotechnology (Omokolo *et al.,* 1995). Nowadays the production of *X. sagittifolium* minitubers can be considered as a revolution, like the production of potato minitubers. *Xanthosoma sagittifolium* minitubers could be used as an alternative for basic seeds produced through plantlets obtained *in vitro* (Djeuani *et al.,* 2014).

Inspite of the important role played by cocoyam in feeding, another major factor which accounts for low production is the increasing decline in soil fertility levels and lack of soil management practices for continuous cocoyam cultivation (Agbede and Adekiya, 2016). Low activity clays characterize tropical soils and the magnitude of nutrient depletion especially in Africa's agricultural production systems is enormous (Stoorvogel and Smaling, 1990). This depletion of soil fertility is widely recognized as the major cause of low food crop production in sub-Saharan Africa (Sanchez, 2002). External fertilising agents to agricultural production systems include mineral fertilisers such as urea, NPK, ammonium nitrate, sulphates, and phosphates; organic fertilisers such as animal manures, composts, and

biosolids. The use of mineral fertilizers in sub-Saharan Africa is limited by the lack of purchasing power and scarcity of the product in the smallholder sectors while their continuous use can also lead to a decline in soil organic manure (SOM) by enhancing its decomposition (Giller *et al.,* 2009), making SOM a critical nutrient source. The use of organic and mineral fertilizers are the two major and common ways in which soils are managed since the extinction of shifting cultivation as well as reduction in bush fallow periods (Makinde *et al.,* 2011). The impact of increased use of mineral fertilizers on crops has been high but the resulting soil physical degradation, increased soil acidity and soil nutrient imbalance, resulting in reduced crop yield (Ojeniyi, 2000; Mbah and Mbagwu, 2006), escalating cost and unavailability of mineral fertilizers (Suge *et al.,* 2011) have drawn the attention of researchers back to the use of organic manures. Organic manures contain humic substances which play a vital role in soil fertility and plant nutrition. Plants grown on soils which contain adequate humin, humic adds (HAs), and fulvic adds (FAs) are less subject to stress, are healthier, produce higher yields; and the nutritional quality of harvested foods and feeds are superior. These organic manures like poultry manure are cheaper, readily available and affordable for soil fertility management and improvement in crop yield. The availability of inorganic nitrogen in particular has the potential to influence the synthesis of secondary plant metabolites, proteins, and soluble solids (Banerjee *et al*., 2012). Different manures (fish, pigeon and cow) and synthetic fertilizers (nitrogen) have been shown to influence some biochemical activities like phenolic constituents of plants (Tugha *et al.,* 2014). The quantity of phenolic or polyphenolic compounds present in a given species of plant material varies with a number of factors such as cultivar, environmental conditions, cultural practices postharvest storage and processing (Chandrasekara and Kumar, 2016). Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plant fruits, leaves, grains, barks, roots, stems and flowers (Panche *et al*., 2016; Rana and Gulliya, 2019) and could also serve as chemotaxonomic marker compounds. Over 9000 flavonoids have been reported (Wang *et al*., 2011). Flavonoids are frequently found as glycosylated or esterified forms, consisting of C6-C3-C6 rings, namely rings A and B linked by three carbon-ring C (Rana and Gulliya, 2019). According to substitution pattern variations, flavonoids can thus be classified into different subclasses, providing an extremely diverse range of derivatives (Tian-yang *et al.,* 2018). Due to their physical and biochemical properties, they are able to participate in plants' interactions with other organisms (microorganisms or animals) (Rana and Gulliya, 2019) and other plants (Erica *et al.,* 2017) and their reactions to environmental stresses (Takahashi and Ohnishi,

2004). Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV-filter, function as signal molecules, allelopathic compounds (Samanta *et al.,* 2011), phytoalexins, detoxifying agents (Dixon, 2010) and antimicrobial defensive compounds (Harborne, 2000). The majority of their functions result from their strong antioxidative properties (Justyna *et al.,* 2014). From the afore mentioned functions it could be suggested that flavonoids play significant roles in plant growth. Flavonoids are also believed to have various bioactive effects including anti-viral, anti-inflammatory, cardio protective, anti-diabetic, anti-cancer, anti-aging, in humans (Tian-yang *et al.,* 2018). Some flavones like vitexin and isovitexin are active components of many traditional Chinese medicines, and were found in various medicinal plants. Vitexin (apigenin-8-C-glucoside) has recently received increased attention due to its wide range of pharmacological effects, including but not limited to anti-oxidant, anti-cancer, anti-inflammatory, anti-hyperalgesic, and neuroprotective effects. Isovitexin (apigenin-6-C-glucoside), an isomer of vitexin, generally purified together with vitexin, also exhibits diverse biological activities (He *et al.,* 2016). Faisal *et al.* (2014) conducted oral glucose tolerance and antinociceptive activity tests with methanol extracts (containing apigenin and its sugar dervatives) from *Xanthosoma violeceum* aerial parts and concluded that bioactive components in these extracts like apigenin, vetexin and isovetexin could be used to lower blood sugar levels in diabetic patents and alleviate pain. Ntengna *et al.* (2019) also identified derivatives of apigenin in cocoyam (*Xanthosoma sagittifolium*) leaves. *X. sagittifolium* leaves contain significant antioxidant compounds which have anti-diabetic properties (Engelberger *et al.,* 2008) and can also be used to treat gastro-intestinal illnesses (Aguegia *et al*., 2000).

Xanthosoma sagittifolium responds very well to input of fertilizer whether organic or inorganic as reported by several authors (Hota *et al.,* 2014; Ogbonna and Nwaeze, 2012; Ojeniyi *et al*., 2013). It has a high requirement for potassium like all other tuber crops (Obigbesan, 1980). In tuber crops, potassium plays a vital role in the movement of sugars produced in the leaf by photosynthesis to the tubers where the sugars are converted to starch (Al-Moshileh *et al.,* 2005; Abd El-Latif *et al.,* 2011).

Against the above background, our research hypothesis was that; cocoyam minitubers can serve as seeds to farmers. The general objective of this study was to evaluate the field performance of cocoyam (*Xanthosoma sagittifolium* L. Schott**)** (white and red cultivars)

minitubers under different fertilizer soil amendments (Poultry manure and NPK 20:10:10). Specific objectives were to:

- ✓ evaluate some soil physico-chemical properties and agro-morphological parameters of cocoyam during culture under different fertilizer soil amendments;
- ✓ evaluate the effect of different fertilizer soil amendments on the content of some mineral nutrients in the cocoyam tubers harvested;
- ✓ determine the effect of different fertilizer soil amendments on the activities of some enzymes, and on content and profiles of some secondary metabolites in cocoyam leaves during growth.

CHAPTER 1: LITERATURE REVIEW

1.1.Generalities on cocoyam

1.1.1. Origin and geographical location

Cocoyam *(Xanthosoma sagittifolium L*. schott), is a herbaceous plant that originated from tropical America and its cultivation dates from the pre-Columbian period (Nzietchueng, 1985; Omokolo *et al.*, 1998). This plant was introduced to Central and West Africa between the sixteenth and seventeenth centuries by the Portuguese slavers into Sao Tomé and Principé (Brown, 2000). The introduction of *X. sagittifolium* in Asia and Oceania occurred in the nineteenth century as a result of missionaries' intervention (Wilson, 1984). This plant is adapted to tropical and subtropical ecosystems with a mean optimum growth temperature. Today, the culture of *X. sagittifolium* is widely spread and covers West and Central Africa, South and Central America, South-East Asia, the Caribbean, Oceania and New Caledonia (Giacometti and Léon, 1994; Brown, 2000).

Worldwide, cocoyam *(Xanthosoma sagittifolium L*. schott) is among the six most important root and tuber cultivated crops. The world production of cocoyam is estimated at 0.45 million tonnes with three fourths coming from Africa (Anonymous, 2006). About 37 % of world's production comes from Nigeria (Anonymous, 2007). In Cameroon, it occupies the second position after cassava (*Manoic esculenta)* in terms of tuber production. (Onokpise *et al.*, 1999 ; Brown, 2000). Its cultivation takes place in seven out of the ten regions of the country. Its culture is found between the 2^{nd} and the 9^{th} degree latitudes North where it is practiced traditionally (Agueguia, 1991; Schafer, 1999). This crop is usually planted together with other crops like maize, cassava, cocoa and banana.

I.1.2. Botanical description

Cocoyam is a herbaceous plant with an annual cycle. The adult plantlet can attain a height of 2 m. It is made up of two main parts; an aerial portion and a subterranean portion.

I.1.2.1. Aerial portion

The aerial portion of cocoyam has a short stem, which bears a cluster of 7-8 large, green, alternate, sagittate (arrow-shaped) leaves. These leaves are borne on long ribbed petioles. The petioles are encased in each other. The superior surfaces of the leaves are

smooth while veins ramify the inferior surfaces of the leaves. These veins are ensheathed at the bases of the leaves (Fig.1).

Flowering is naturally rare but, can be induced by the application of gibberellic acid (GA_3) on the plants at the stage of 5 to 6 leaves. Inflorescences sprout between the leaves in a cylindrical spadix. The spadix has a white spathe of about 12 to 15 cm. This spathe closes at the base in the form of a spherical chamber and opens at the top in a concave lamina. The spathe bears female flowers on its lower portion, male flowers on its upper portion and sterile flowers in the middle (Giacometti et Léon, 1994). At maturity the aerial portion is usually reduced.

I.1.2.2. Subterranean portion

This portion has a mother tuber or corm (rhizome), which gives rise to the principal tuber. Swollen secondary tubers sprout laterally from the principal tuber. The principal tuber also bears numerous roots. The principal tuber, which has a length of about 15 to 25 cm, is larger towards the apex (in the direction of the soil) (Agueguia *et al.*, 2000).

Fig.1. Xanthosoma sagittifolium plant: white cultivar (age: 6 months) (scale = 2cm) L: Leaf, P: Petiole, R: Rhizome (photo Gwan, 2018).

I.1.3. Classification and genetic diversity

Watson and Dallwitz (1997) proposed a revised taxonomy of *X. sagittifolium* as follows:

Kingdom : Plantae

Phylum : Magnoliophyta

Class : Liliopsida

Sub-Class : Arecidae

Super-order : Spadiciflores

Order : Arales

Family : Araceae

Sub-family : Colocasioidae

Tribe : Caladieae

Genus : Xanthosoma

Species : *Xanthosoma sagittifolium*

The Araceae family includes close to 110 genera and more than 2,500 species (Brown, 2000). It is subdivided into nine sub-families and both taro and cocoyam belong to the Colocasioideae sub-family. *Xanthosoma* is a genus of the Araceae family with about 50 species (Thompson and Dewet, 1983). Their distinctive traits (size of leaves, venation, colour of petiole and tuber colour) are not clearly defined (Stephens, 1994). Depending on the morphological aspect (colour of tuber flesh and hardness of tuber), the ploidy, the productivity and the resistance to *Pythium myriotylum*, *X. sagittifolium* is classified into three cultivars in cameroon:

- The white very productive cultivar, with tender tubers and diploid cells (2n= 26 chromosomes) which is sensitive to root rot;
- The red productive cultivar with hard tubers and diploid cells (2n= 26 chromosomes) which is senstive to root rot

- The yellow less productive cultivar with tender tubers and tetraploid cells, (4n= 56 chromosomes) which is resistant to root rot (Ngouo, 1988).

I.1.4. Ecology and reproduction

X. sagittifolium is an annual plant with a growth cycle lasting between 9 and 11 months (Ngouo, 1988) depending on the cultivar and growth conditions. *X. sagittifolium* is a tropical and sub-tropical rainforest plant cultivated between latitude 30^{o}North and15^{o}South. In Cameroon it grows under the forest canopy in association with other plants such as cocoa, banana, plantains and palms. It requires optimum temperature ranges between 20 - 35 °C and rainfall between 1500 and 3000 mm. It also requires well-drained soils with pH varying between 5.5 and 6.5 and does not tolerate permanent presence of water (Agueguia *et al.*, 2000).

Its reproduction takes place principally through vegetative propagation, whereby planting material consist mainly of tuber and rhizome fragments or whole tubers (Agueguia *et al.*, 2000). The plant development consists of three major stages which are plant establishment (from planting to about 2 months after planting), a rapid vegetative growth (2 to 5 months after planting), tuber development and maturation (after 5 or 6 months) (Asumadu *et al.*, 2011). The senencence of leaves during the third stage is an indicator of harvest to farmers.

I.1.5. Importance

The importance of cocoyam (*X. sagittifolium*) is mainly nutritional. This plant is also used traditionally in different localities for the treatment of illnesses (Esso *et al.*, 1987; Kingue *et al.*, 1994) and it is also of economic importance to farmers and industries.

I.1.5.1. Nutritional importance

Xanthosoma sagittifolium provides about one third of food intake for approximately 200 million people in the tropics and subtropics and for more than 400 million people worldwide (Onokpise *et al.*, 1999). The edible tubers and the young leaves constitute a good source of carbohydrates, some proteins, vitamins, some mineral salts and fats (Sefa-Dedeh and Agyir-Sackey, 2004; Ndabikunze *et al.*, 2011) (Tables 1 and 2). Its fresh cocoyam corms and cormels contain high calcium oxalate crystal 780 mg.100 g^{-1} in some species (Sanful and Darko 2010) which is irritating to the skin and the mouth. This effect of corm and cormel is removed when cooked. Cocoyam also constitutes the staple food for populations due to its

richness in nutritive elements capable of covering the quasi totality of energy need and up to 60 % of protein need of an adult (Boudjeko, 2003). Young *X. sagittifolium* leaves contain an average of 20 % protein while the tubers have 2-3 % protein, 15-39 % carbohydrates and 77 % water (Agueguia *et al.,* 2000). This plant can also be used to feed domestic animals like pigs for instance, due to its leaves; especially when they are still green at harvest and the main tuber that is very fibrous (Rodriguez *et al.*, 2009).

In Cameroon, cocoyam is prepared, processed, and consumed in many forms by ethnic groups (Tandehije, 1990). These forms include cormels peeled, boiled and eaten with vegetable soap, *ekwang* and *kwacoco*.

Table 1: Nutritional composition of *X. sagittifolium* per 100g of fresh matter

Class of food	Water (g)	Energy Kcal	Glucides (g)	Proteins (g)	Lipids (g)	Ca (mg)	Fe (mg)	VitA (UI)	VitB1 (mg)	VitB2 (mg)	VitC (mg)	Nicotinic acid (mg)
Content	58-78	133	31	2.0	0.3	20	1.0	Traces	0.01	0.03	10	0.5

Source: Mbahe *et al.* (2002).

Table 2: Mineral content of *X. sagittifolium* per 100g of dry matter

Mineral	P	K	Cu	Fe	Zn	Mn	Ca	Mg	Na
Content(mg)	207.50	908.25	0.63	4.54	2.72	90.62	110.17	90.62	23.98

Source: Ndabikunze *et al*.(2011).

I.1.5.2. Medicinal importance

Medicinally, consumption of *X. sagittifolium* tubers helps to enhance the regulation of the acido-basic equilibrium in the human body (Njoku and Ohia, 2007). The rich content in iodine (79.5ug.100g^{-1}) in fresh tubers can be used to fight diseases due to iodine deficiencies in children and pregnant women (Taga *et al*., 2004). *X. sagittifolium* leaves contain significant antioxidant compounds which have anti- diabetic properties (Engelberger *et al.,* 2008) and

can also be used in treatment of some gastro-intestinal illnesses or allegies (Agueguia *et al.*, 2000).

In Cameroon, its young leaves are used for the treatment of allergies and gastro-intestinal illnesses. Esso *et al.* (1987) and Kingue *et al.* (1994) brought out the illnesses and the different parts of the plant used to treat each illness traditionally in the Douala and Abo localities.

Table 3: Cocoyam (*X. sagittifolium***)** plant parts used in the Douala and Abo traditional pharmacopies.

Illness	Eye problems	Panaris	Abscess	Jaundice	'Frontanelle'
Plant part used	Tubers	Young leaves	Young leaves and tubers	tubers	leaves

Sources: Esso *et al.*(1987); Kingue *et al.*(1994).

I.1.5.3. Economic importance

Africa is the first producer of *X. sagittifolium* with 37% of the world's production coming from Nigeria (Anonymous, 2006). Studies have revealed that in producer countries, *X. sagittifolium* culture is mostly carried out by women in small family plantations mainly for household consumption and the excess sold in the local markets. Cocoyam (*X. sagittifolium*) tubers are largely used by food industries and this makes up part of the new exportation products of the producing countries (Onyenweaku and Okoye, 2007). Cocoyam (*X. sagittifolium*) flour is one of the few processed forms of cocoyam (*X. sagittifolium*) encountered in Africa. This is made by peeling and slicing fresh or cooked corms and cormels, drying the slices and milling the dried slices into flour. The cocoyam (*X. sagittifolium*) flour is used in many localities as supplements for the production of cakes and bread with more organoleptic properties compared to that of a 100 % wheat flour (Sanful and Darko 2010). The flour is also used for the preparation of soups, biscuits, breverages and puddings (Ndabikunze *et al.*, 2011) and also alcohol production (Adelekan, 2012).

I.1.6. Production problems

Despite the important role played by *X. sagittifolium* in feeding, its culture and production is still faced with many obstacles which include:

I.1.6.1. Poor traditional culture methods.

Xanthosoma sagittifolium seeds are mostly produced through vegetative propagation. These seeds are either new tubers or tuber fragments. This limits the number of planting materials because only one corm is planted and recycled while the cormels used in planting are those that have developed leaves or are exposed to the atmosphere. These poor traditional methods do not prevent, disease transmission from one culture generation to another, hence reducing production (Schafer, 1999; Agueguia *et al.,* 2000).

I.1.6.2. Parasitic infections

The numerous parasite attacks on cocoyam (*X. sagittifolium*) result in a fall in quality and quantity of seeds. Among the parasitic agents of cocoyam is *Pythium myriotylum* which causes root rot. Root rot is the major obstacle to cocoyam cultivation. In Cameroon, *P. myriotylum* is the first cause in yield falls (Wutoh *et al.*, 1991). In certain fields, this fungus can account for up to 90 % of yield falls (Pacumbaba *et al.*, 1992; Tambong et Hofte, 2001). In addition to this pathogen we have *Leptosphaeulina trifolii* that causes leaf spots; *Sclerotium rolfsii* which is responsible for callus rot (Lyonga, 1979) and Dashen mosaic virus responsible for tuber rots (Anon, 1991; Chen and Adams, 2001). Root-knot nematods (Meloidogyne spp), cause galls and irregular swellings on the underground portions of cocoyams, resulting in stunting of the plant.

I.1.6.3. Decline in soil fertility levels

The increasing decline in soil fertility levels and lack of soil management practices for continuous cocoyam cultivation is a major factor which accounts for low production (Agbede and Adekiya, 2016). Low activity clays characterize tropical soils and the magnitude of nutrient depletion especially in Africa's agricultural production systems is enormous (Stoorvogel and Smaling, 1990). This depletion of soil fertility is widely recognized as the major cause of low food crop production in sub-Saharan Africa (Sanchez, 2002).

I.1.7. Strategies to ameliorate *X. sagittifolium* production

Many methods are currently being used or under study in order to improve production. Some of them include:

- **New cultural practices**: Like the selection of seeds to prevent disease propagation, the use of organic and mineral fertilizers which are the two major and common ways in which soils are managed since the extinction of shifting cultivation as well as reduction in bush fallow periods (Makinde *et al.,* 2011). Cocoyam responds very well to input of fertilizer whether organic or inorganic with a net increase in foliar development and tuber yield (Hota *et al.,* 2014; Ogbonna and Nwaeze, 2012; Ojeniyi *et al*., 2013).

- **Chemical control**: This involves the use of pesticides to fight against bioagressors but with negative environmental effects and a high cost of chemicals (Khi-Hyun *et al.,* 2017).

- **Biological control**: Here microorganisms which are antagonistic to *Pythium myriotylum* are used. Isolates of *Trichorderma haziarnum* and *Pseudomonas florescens* have shown efficient results against *Pythium myriotylum* but this biological approach is not yet vulgarised (Peernel *et al.,* 2008).

- **Using elicitors to stimulate natural plant defence mechanisms:** Studies have revealed that elicitors like salicylic acid (SA), 1,2,3 Benzo thiadiazole-7-carbothonic acid-s-methyl ester (BTH) (Mbouobda *et al.,* 2011), chitosan(CTH) (Djeuani *et al.,* 2009) stimulate natural plant defence mechanisms. In *X. sagittifolium* chitosan induces oxidative stress in the plant slows down mycelia growth of *Pythium myriotylum* (Djeuani *et al.,* 2009; Mbouobda *et al.,*2011).

- **Application of plant biotechnology:** The use of biotechnological methods mainly tissue culture could be a useful alternative for the production of pathogen-free plants, development of new varieties, mass production of planting material and the conservation of germplasms (Omokolo *et al*., 2003). In *X. sagittifolium* tissue culture has led to the production of vitroplants and microtuberization (Tsafack *et al*., 2009). Minituberization produces cocoyam

minitubers from acclimatised vitroplants and these minitubers can be used as an alternative to basic seeds (Djeuani *et al*., 2014).

- **Use of arbuscular mycorrhizae:** The use of arbuscular mycorrhizae in improving growth and production in plants is increasing. Mycorrhizal strains of *Glomus intraradices* and *Gigaspora margarita* have significantly influenced growth and minituberization in cocoyam (Djeuani *et al*., 2017).

I.2.Tuberization

I.2.1. Definition

Tuberization is the process of differentiation of the subterranean portion of a plant into a reserve organ called tuber (Hajrezaei *et al.*, 2000) and involves modifications which accompany the transformation of the stem, root or hypococytl into a storage organ (tuber, rhizome, bulb and tuberized root) (Djeuani, 2018).

Tuber plants show different modes of tuber formation, for example in *Solanum tuberosum* the tuber originates from a stolon (Du Jardin, 1994). In *X. sagittifolium*, *Colocasia esculenta*, and *Dioscorea spp.*, tubers are derived from meristematic zones of rhizomes while in *Manihot esculenta* tubers are formed from enlarged roots.

I.2.2. Methods of tuberization

1.2.2.1. *In vitro* tuberization or micotuberization

In vitro tuberization is the production of tubers in test tubes. The tubers obtained are called microtubers. These tubers produced from vitroplants, are reduced in size, have small masses but possess a good germination capacity (Akogo *et al.*, 1995). Microtubers offer several advantages. They are convenient to handle, transport and store germplasm and they can be used as experimental research tools for biochemical, physiological and genetic studies of plants (Colemann *et al.*, 2001). *In vitro* tuberization is one of the strategies being developed to solve the problems of availability of disease-free seeds for the farmer in all the seasons. This technique has been applied to other crops like the potato (*Solanum tuberosum*) (Sidikou *et al.*, 2005).

1.2.2.2. Minituberization

In minituberization, tubers are formed from acclimatized vitroplants in greenhouse or under a shed. In green houses, factors affecting tuberization like light, water, temperature, hormones and fertilizing agents can be controlled. The tubers obtained are called minitubers and are average in size compared to tubers produced in the field. These tubers are not used for food, but, could be used as seeds (Djeuani *et al.,* 2014, 2018). The seeds are disease-free, have universal applications, interesting for industrial production (Dowling, 2000) and can be produced without seasonal constraints.

1.2.2.3. Field tuberization

Field tuberization is the accumulation of products of photosynthesis like sugars in reserve organs, under natural conditions. Here, the plant grows in an environment where it is not possible to control factors that influence tuberization. Cocoyam tubers obtained through this method are generally large and destined for food. Production is limited by poor culture methods and parasitic diseases (Chen and Adams, 2001).

I.2.3. Factors influencing tuberization

I.2.3.1. Edaphic factors

Edaphic factors are soil related factors. Plants obtain most of their oxygen and carbon from the air by photosynthesis and hydrogen is obtained directly or indirectly, from the water in the soil (Green *et al.,* 2002). These three elements together make up over 90 percent of fresh plant tissue. However, plants cannot survive without the much smaller quantity of essential nutrients that they obtain from the soil, such as nitrogen, phosphorus, potassium, calcium, magnesium and sulphur. Soil also provides the place for roots to anchor and grow. It holds the water in which the soil nutrients are changed into ions, which is the form that the plant can use. It holds the air that prevents the plants from becoming waterlogged. It holds the chemicals that determine the soils pH, salinity and dispersitivity (Raymond and Brady, 2016). The main components of soil include;

- **Inorganic particles**; the inorganic portion of soil is derived from solid rock (bedrock) through weathering processes and are made up of small rock fragments and minerals. These inorganic particles are classified by size as gravel or stone, sand, silt, or clay (Table 4). The size of the inorganic particles influences soil texture

Table 4: Rock fragments and mineral particles classified by size (Buckman and Brady, 1960).

Common name	**Size Description**	**Diameter**	**Feel**
Gravel, stone	Very coarse	Larger than 2mm	Rocky
Sand	Coarse	0.02 mm to 2 mm	Gritty
Silt	Fine	0.002 mm to 0.02 mm	Smooth, silky
Clay	Very fine	Smaller than 0.002 mm	Sticky, plasticine

- **Organic matter;** this portion of the soil is derived from decaying residues of plants and animals (living organisms). The major roles of organic matter in soil are adding nutrients and improving the soil's structure and water-holding capacity. Soils with low organic matter have 'poor' structure, poor water holding capacity, and erode or leach nutrients easily. Soils with high organic matter levels have 'good' structure, good water-holding capacity, and reduced erosion and nutrient leaching. When the organic matter is fully broken down, one of the things that is left is humus. Much of the humus is formed from the lignin in plants. Lignin is essentially indigestible by living organisms, and this is part of the reason why humus remains in the soil long after the other plant matter has decayed away (for several thousands of years). Humus ranges in colour from brown to black, and the intensity of its colour is influenced by climate (rainfall and temperature) rather than by the amount of organic matter in the soil (Raymond and Brady, 2016).

I.2.3.1.1. Physical properties of soil

Physical properties of a soil that affect a plant's ability to grow include:

- Soil texture, which affects the soil's ability to hold onto nutrients (cation exchange capacity) and water. Texture refers to the relative distribution of the different sized particles in the soil. It is a stable property of soils and, hence, is used in soil

classification and description. Soil texture, or the 'feel' of a soil, is determined by the proportions of sand, silt, and clay in the soil. When they are wet, sandy soils feel gritty, silty soils feel smooth and silky, and clayey soils feel sticky and plastic, or capable of being moulded (Table 4). Soils with a high proportion of sand are referred to as 'light', and those with a high proportion of clay are referred to as 'heavy'. The names of soil texture classes are intended to give you an idea of their textural make-up and physical properties. The three basic groups of texture classes are sands, clays and loams. A soil in the sand group contains at least 70% by weight of sand. A soil in the clay group must contain at least 35% clay and, in most cases, not less than 40%. A loam soil is, ideally, a mixture of sand, silt and clay particles that exhibit light and heavy properties in about equal proportions, so a soil in the loam group will start from this point and then include greater or lesser amounts of sand, silt or clay. Additional texture class names are based on these three basic groups. The basic group name always comes last in the class name. Thus, loamy sand is in the sand group, and sandy loam is in the loam group (Fig. 2) (Buckman and Brady, 1960).

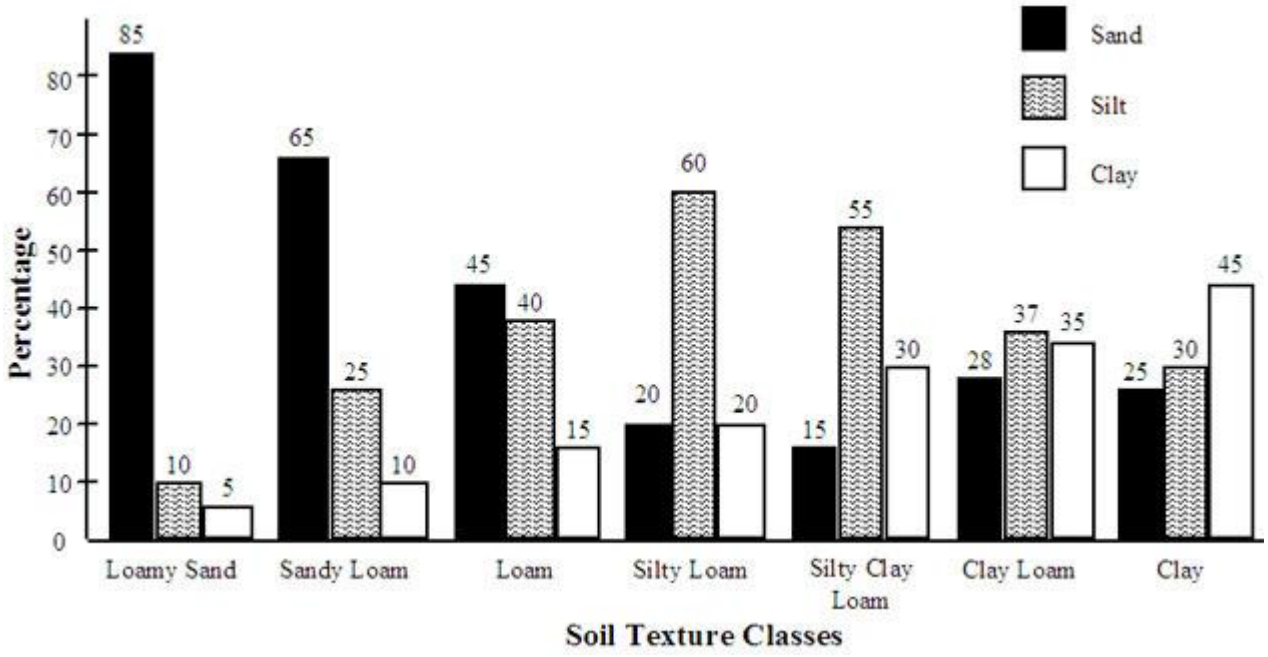


Fig. 2. Soil texture classes (Buckman and Brady, 1960)

- Soil structure, which affects aeration, water-holding capacity, drainage and penetration by roots and seedlings among other things. Soil structure refers to the arrangement of soil particles into aggregates (or peds) and the distribution of pores in between

(Buckman and Brady, 1960). It is not a stable property and is greatly influenced by soil management practices.

I.2.3.1.2. Chemical properties of soils

The chemical properties of soils that are important to plant growth and development are:

- Nutrient availability and cation exchange capacity, which affect the soil's inherent fertility and its ability to hold nutrients.
- The chemical characteristics of the soil solution, which affect pH and salinity.
- The sodicity of the soil, which affects soil stability (Raymond and Brady, 2016).

I.2.3.2. Nutritional factors

Amongst the nutritional factors which influence tuberization, nitrogen and the carbon sources are cited to be the most important in the culture medium:

Nitrogen: The nitrogen intake constitutes the primordial factor in the process of tuberization (Charles and Rossignol, 1992) for it plays at the same time structural and functional roles. Nitrogen is one of the 15 essential elements that plants require (Marschner, 1986). It also enters in the constitution of many important molecules like amino acids, amides, proteins, nucleic acids, nucleotides and certain hormones. This macroelement favours vegetative growth and increases the green coloration linked to the abundance of chlorophyll. Nitrogen tends to prolong the functional duration of vegetative organs and retards maturation and senescence (El-Hassani and Persoons, 1994). Plants do not take-in nitrogen in the free state (N), but, with the help of nitrifying bacteria where it is converted to NO_3^- and NH_4^+. The ratio NO_3^-: NH_4^+ significantly affects the process of tuberization. Omokolo *et al.* (2003), have demonstrated that the ratios 1:1 and 2:1 permit the obtention of high number of microtubers.

Sugars: sugars are the main products of photosynthesis. In addition to their structural role, sugars (carbohydrates) provide the organism with its energetic needs when oxidized. Sugars influence *in vitro* tuberization by their nature and the quantity present in the culture medium. Sucrose is the main form in which synthesized sugars are translocated in plants. It intervenes in tuberization by inducing the synthesis and accumulation of starch in the tubers (Scofield *et al.*, 2007). In *X. sagittifolium* high sucrose concentrations (30%) in the culture medium induces tuberization even in the absence of other growth substances (Tsafack *et al.*,

2009; Djeuani *et al.,* 2014). Maltose is a disaccharide made up of two glucose sub units and can be obtained from the hydrolysis of starch. In *Solanum tuberosum* maltose favours the formation of tubers with reduced sizes and masses (Du Jardin *et al*., 1994) while in *X. sagittifolium* plantlets treated with maltose solutions during growth and minituberization produce more leaves that plantlets treated with sucrose of the same concentrations (Abilogo, 2013). Again after 171 days of culture the minituber sizes are larger in plants treated with maltose than those treated with sucrose (Djeuani *et al.,* 2014).

I.2.3.3. Phytohormonal factors

Plant growth regulators are natural organic substances which influence most physiological processes at low concentrations, growth regulators play a complex role in the tuberization process. They probably assure the transmission of environmental stimuli to the different levels of organization of the plant and condition the receptivity of plant tissues and cells to these stimuli (Suttle, 2000). Growth regulators condition the plant organs to emit, transmit and interpret primary signals of tuberization. According to Colemann *et al.* (2000) and Guivarc'h *et al.* (2002), phytohormones like auxins, cytokinins, Abscissic acid and ethylene play a role in the induction of tuberization.

I.2.3.4. Environmental factors

In vitro tuberization is mostly influenced by the photoperiod and thermoperiod. The main external factor is the photoperiod, which is defined as the length of daily illumination. Long day plants require at least 9 hours of daily illumination while short day require less than 9 hours in order to perform a particular physiological process like flowering (Clegg and Mackean, 2000). In all tuberous plants there exist a critical photoperiod above which tuberization is inhibited (Alleman and Hammes, 2006). The quantity and quality of light received by the plant influences the synthesis of constitutive products of tubers (Charles and Rossignol, 1992). A change in photoperiod stops growth and induces tuberization. Rodriguez *et al.* (2006) have demonstrated that the inductive stimulus is produced at the level of the leaves and translocated to the basal parts. According to Allemann and Hammes (2006), this stimulus is activated by photoperiods lower than the critical photoperiod of the plant. Short-day (SD) favours early tuberization and this reaction of SD is relative as a function of varieties and/or species (Sidikou *et al.,* 2003).

Temperature also affects tuber formation. Low temperatures favour tuberization while high temperatures retard it. According to Omokolo *et al*., 2003 it is necessary to have a variation in temperature between the luminuous period (26 °C) and the dark period (20 °C) in order to trigger tuberization in *X. sagittifolium.* In *Solanum tuberosum* optimum temperatures for tuberization lie between 15 °C and 18 °C (Akita and Takayama, 1994).

I.3.Generalities on fertilizers

I.3.1. Definition

A fertilizer can be considered to be any substance which is added to soils or to plant tissues to supply one or more nutrients essential to the growth of plants. Fertilizers can either be made up of inorganic substances (chemical fertilizers), organic substances (manures) and beneficial microorganisms (biofertilizers) (Yumbi *et al*., 2017).

I.3.2. Types of fertilizers

I.3.2.1. Organic fertilizers

These are fertilizers which originate from animal or plant waste matter. Organic fertilizers slowly release nutrients to plants, are non-toxic to environment and not easily leached from the soil, examples include:

- **Farm manure,** which consist of animal excreta like poultry manure, cow dung, pig dung, goat manure etc.
- **Compost,** consisting of a mixture of organic wastes that have allowed to ferment.
- **Green manure,** which is made up of plant residues in the farm that are buried to increase soil fertility.
- **Vermicompost,** which are composts produced using earthworms (Yumbi *et al*. 2017).

I.3.2.1.1. Use of farm manure in tuber production

Organic manures like farm manures contain humic substances which play a vital role in soil fertility and plant nutrition. Plants grown on soils which contain adequate humin, humic adds (HAs), and fulvic adds (FAs) are less subject to stress, are healthier, produce higher yields; and the nutritional quality of harvested foods and feeds are superior. These

organic manures like poultry manure, cow dung, pig dung are cheaper, readily available and affordable for soil fertility management and improvement in crop yield. *Xanthosoma. sagittifolium* responds very well to input of fertilizer whether organic or inorganic as reported by several workers (Hota *et al.*, 2014; Ogbonna and Nwaeze, 2012; Ojeniyi *et al*., 2013). Hamma *et al.*,2014 showed that goat manure, cow dung and poultry manure significantly increased yields in *Colocasia esculenta*. Cow dung treatments have been shown to increase the organic carbon, organic matter and exchangeable cations in the soil, thereby improving potato (*Solanum tuberosum*) tuber yield (Suh *et al.*,2015).

I.3.2.2. Inorganic fertilizers

These are fertilizers which are derived from chemical products. They can be used to make the desired ratio of nutrients and are easy to apply. Their applications can lead to leaching, soil acidity, toxicity to soil organisms and harm to the environment (Ojeniyi, 2000; Mbah and Mbagwu, 2006), Some examples include:

- **Nitrogenous fertilizers** like sodium nitrate, ammonium nitrate, potassium nitrate.
- **Phosphatic fertilzers** like ammonium phosphate.
- **Potassic fertilizers** like potassium ntrate, potassium sulphate, potassium chloride.
- **Mixed and compound fertilizers** like NPK 20:10:10, NPK 15:15:15, NPK 10:20:20 etc (Yumbi *et al*. 2017).

I.3.2.2.1. Methods of fertilizer application

Different methods can be used to apply fertilizers. Fertilizers can be applied ether in solid or liquid form. Some of these methods are:

- **Broad casting;** involves throwing the fertlizers all over the tilled land. This method is easy but wastes fertilizer.
- **Roll placement;** here a small gutter is made alongside a line of plants and the fertlizer burried. It is easy but time wasting.
- **Ring application;** with this method fertilizer is placed round the plant. There is no waste of fertilizer but it is time consuming.

- **Banding;** fertilizer here is placed round groups of plants.

- **Irrigation;** here fertilizer is put in water and used to irrigate plants. This method is easy but can lead to excess or limited application.

- **Foliar application;** fertilizer is sprayed on plant leaves. Easy to apply but difficult for tall plants (Yumbi *et al*., 2017).

I.3.2.2.2. NPK fertilizers and tuber production

NPK fertilizer is usually used to increase the growth of a plant. The N element in NPK fertilizer has the function of preparing amino acids (proteins), nucleic acids, nucleotides, and chlorophyll in plants. The element P in the NPK fertilizer has a function as a storage and energy transfer. The K element in the NPK fertilizer serves as an enzyme activator, and assists in the transport of assimilated results from the leaf to the plant tissue (Yahya *et al.,* 2018). The impact on growth and yield of tuber crops like *Colocasia esculenta*, *Solanum tuberosum* and *Xanthosoma sagittifolium* from the application of mineral fertilizers like NPK fertilizers is significant (Ogbonna and Nweze, 2012; Suh *et al.,* 2015). In tuber crops, potassium plays a vital role in the movement of sugars produced in the leaf by photosynthesis to the tubers where the sugars are converted to starch (Abd El-Latif *et al.,* 2011). Irish Potatoes (*Solanum tuberosum*) require high amounts of potassium (K) and nitrogen (N) fertilizers for optimum growth, production and tuber quality (Al-Moshileh *et al.,* 2005).

I.3.2.3. Biofertilizers

Fertilizers which are made up of beneficial microorganisms are called biofertilizers. Biofertilizers improve soil structure and texture, increase the rate of nutrient release by soil, and increase the amount of soil nitrogen leading to increased soil fertility. Examples of biofertilizers are Effective microorganisms (EM), Indigenous Microoorganisms (IMO), Arbuscular mycorrhizal fungi (AMF), Nitrogen fixing bacteria etc (Yumbi *et al*. 2017).

I.3.2.3.1. Biofertilizers and tuber production

Studies have shown that Arbuscular mycorrhizal fungi (AMF) are strongly involved in the acquisition of important nutrients of plant in soil. AMFs play a key role in improving the uptake of water in plants, contribute to the increase in antioxidant activity, to osmotic adjustment, improve hormone regulations, biological control of plant pathogens, soil fertility,

plant nutrition, improve absorption and translocation of mineral nutrients. These mineral nutrents are mainly Phosphorus (P), Nitrogen (N), Sulfur (S), Potassium (K), Calcium (Ca), Iron (Fe), Copper (Cu) and Zinc (Zn) and various trace elements from the soil to host plants, due to an extensive hyphae network of the soil, which propagates from roots colonized in the soil (Marulanda *et al.,* 2003; Ceccarelli *et al.,* 2010; Hodje and Storer, 2015). The use of arbuscular mycorrhizal fungi as an inoculum in agriculture remains promising and may be very important in addressing phosphorus deficiency problems in some plants that are highly dependent on their presence in the soil, such as plants with roots and tubers among which the most important are: irish potato (*Solanum tuberosum* L.), cassava (*Manihot exculenta*), taro (*Colocasia exculenta* L. Schott) and cocoyam (*Xanthosoma sagittifolium* L. Schott)(Miransari *et al.,* 2009; Dechassa *et al.,* 2003). The effect of two species of AMF, *Glomus intraradices* and *Gigaspora margarita* was evaluated on the improvement of growth and tuberization in white cocoyam (*Xanthosoma sagittifolium* L. Schott) and the results obtained showed that cocoyam (*Xanthosoma sagittifolium* L. Schott) is a mycotrophic plant (Djeuani *et al.,* 2017, 2018).

I.4. Secondary metabolites in plants

I.4.1 Definition

Secondary metabolites are organic compounds produced by plants which are not directly involved in normal growth, development or reproduction in plants. Plant secondary metabolites can be classified into four major classes: terpenoids, phenolic compounds, alkaloids and sulphur-containing compounds as shown in Table 5 (Guerriero *et al.,* 2018).

Table 5: Major classes of secondary metabolites, sources and some effects.

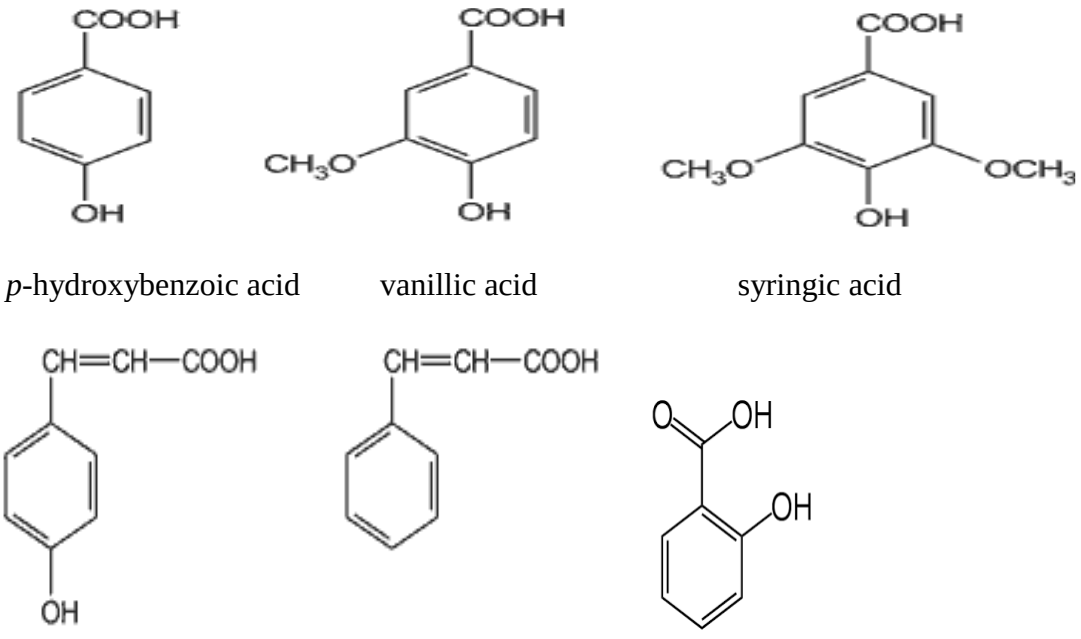
Class	Example Compounds	Example Sources	Some Effects and Uses
NITROGEN-CONTAINING			
Alkaloids	nicotine cocaine theobromine	tobacco coca plant chocolate (cocao)	interfere with neurotransmission, block enzyme action
NITROGEN-AND SULPHUR-CONTAINING			
Glucosinolates	sinigrin	cabbage, relatives	
TERPENOIDS			
Monoterpenes	menthol linalool	mint and relatives, many plants	interfere with neurotransmission, block ion transport, anesthetic
Sesquiterpenes	parthenolid	Parthenium and relatives (*Asteraceae*)	contact dermatitis
Diterpenes	gossypol	cotton	block phosphorylation; toxic
Triterpenes, cardiac glycosides	digitogenin	Digitalis (foxglove)	stimulate heart muscle, alter ion transport
Tetraterpenoids	carotene	many plants	antioxidant; orange coloring
Terpene polymers	rubber	Hevea (rubber) trees, dandelion	gum up insects; airplane tires
Sterols	spinasterol	spinach	interfere with animal hormone action
PHENOLICS			
Phenolic acids	caffeic, chlorogenic	all plants	cause oxidative damage, browning in fruits and wine
Coumarins	umbelliferone	carrots, parsnip	cross-link DNA, block cell division
Lignans	podophyllin urushiol	mayapple poison ivy	cathartic, vomiting, allergic dermatitis
Flavonoids	anthocyanin, catechin	almost all plants	flower, leaf color; inhibit enzymes, anti- and pro-oxidants, estrogenic
Tannins	gallotannin, condensed tannin	oak, hemlock trees, birdsfoot trefoil, legumes	bind to proteins, enzymes, block digestion, antioxidants
Lignin	lignin	all land plants	structure, toughness, fiber

I.4.2. Phenols

Phenolic compounds are plant secondary metabolites playing important roles in plant resistance. Their chemical structure is based on at least one aromatic ring bonded to one or more hydroxyl groups. They are mainly synthesized from amino acid phenylalanine which is converted to cinnamic acid (Kamila, 2016). Phenolics are one of the largest and most diverse groups of plant active substances. These compounds take part in the regulation of seed germination and cooperate in regulating the growth of plants, also taking part in defence responses during infection, excessive sun exposure, injuries and heavy metal stress (Kamila, 2016).

I.4.2.1. Structure and biosynthesis of phenols

All consist of the aromatic ring (C6) bonded directly to at least one (phenol) or more (polyphenol) hydroxyl groups (-OH) and other substituents, such as methoxyl or carboxyl groups which cause the polar character of the compounds and allow dissolution in water (Michalak, 2006). Phenolic compounds are usually divided into two groups; simple phenols and more complex derivatives, often containing several aromatic rings linked together. The group of simple phenols include among others: *p*-hydroxybenzoic acid, *o*-hydroxybenzoic acid (salicylic acid), caffeic acid, gallic acid, vanillic acid, syringic acid, coumaric acid or cinnamic acid. Structure of selected simple phenols are presented at Fig.3 (Kamila, 2016).

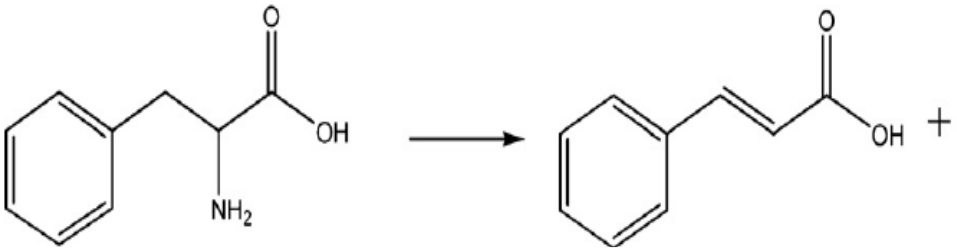


p-hydroxybenzoic acid vanillic acid syringic acid

p-coumaric acid cinnamic acid Salicylic acid

Fig.3. Structures of some simple phenolic compounds

The aromatic ring of phenolic compounds is synthesized in the shikimic acid pathway from amino acid phenylalanine, the common precursor of phenols. The initial step in the formation of all phenolic compounds is simple disengagement of the amino group (-NH2) from phenylalanine which converses to cinnamic acid. The reaction is catalyzed by enzyme Phenylalanine ammonia-lyase (PAL) (Fig.4). Cinnamic acid is then used as the starting molecule for the synthesis of other phenolic compounds (Knaggs, 2003; Bhattacharya *et al*., 2010).



Phenylalanine cinnamic acid

Fig.4. Enzymatic conversion of phenylalanine to cinnamic acid

I.4.2.2. Structure, classification and biosynthesis of flavonoids

Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plant fruits, leaves, grains, bark, roots, stems and flowers (Panche *et al*., 2016) and could also serve as chemotaxonomic marker compounds. Over 9000 flavonoids have been reported (Wang *et al*., 2011). Flavonoids are frequently found as glycosylated or esterified forms, consisting of C6—C3—C6 rings, namely rings A and B linked by three carbon- ring C (Fig. 5). According to substitution pattern variations, flavonoids can thus be classified into different subclasses, providing an extremely diverse range of derivatives (Tian-yang *et al.,* 2018) (Fig.5 and Fig.6). Due to their physical and biochemical properties, they are capable of participating in plants' interactions with other organisms (microorganisms, animals and other plants) and their reactions to environmental stresses. Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV-filter, function as signal molecules, allelopathic compounds, phytoalexins, detoxifying agents, antimicrobial defensive compounds. The majority of their functions result from their strong antioxidative properties (Justyna *et al.,* 2014). From the aforementioned functions it could be suggested that flavonoids play significant roles in the growth and development of most plants. They are also believed to have various bioactive effects

including anti-viral, anti-inflammatory, cardio protective, anti-diabetic, anti-cancer, anti-aging, etc (Tian-yang *et al.*, 2018).

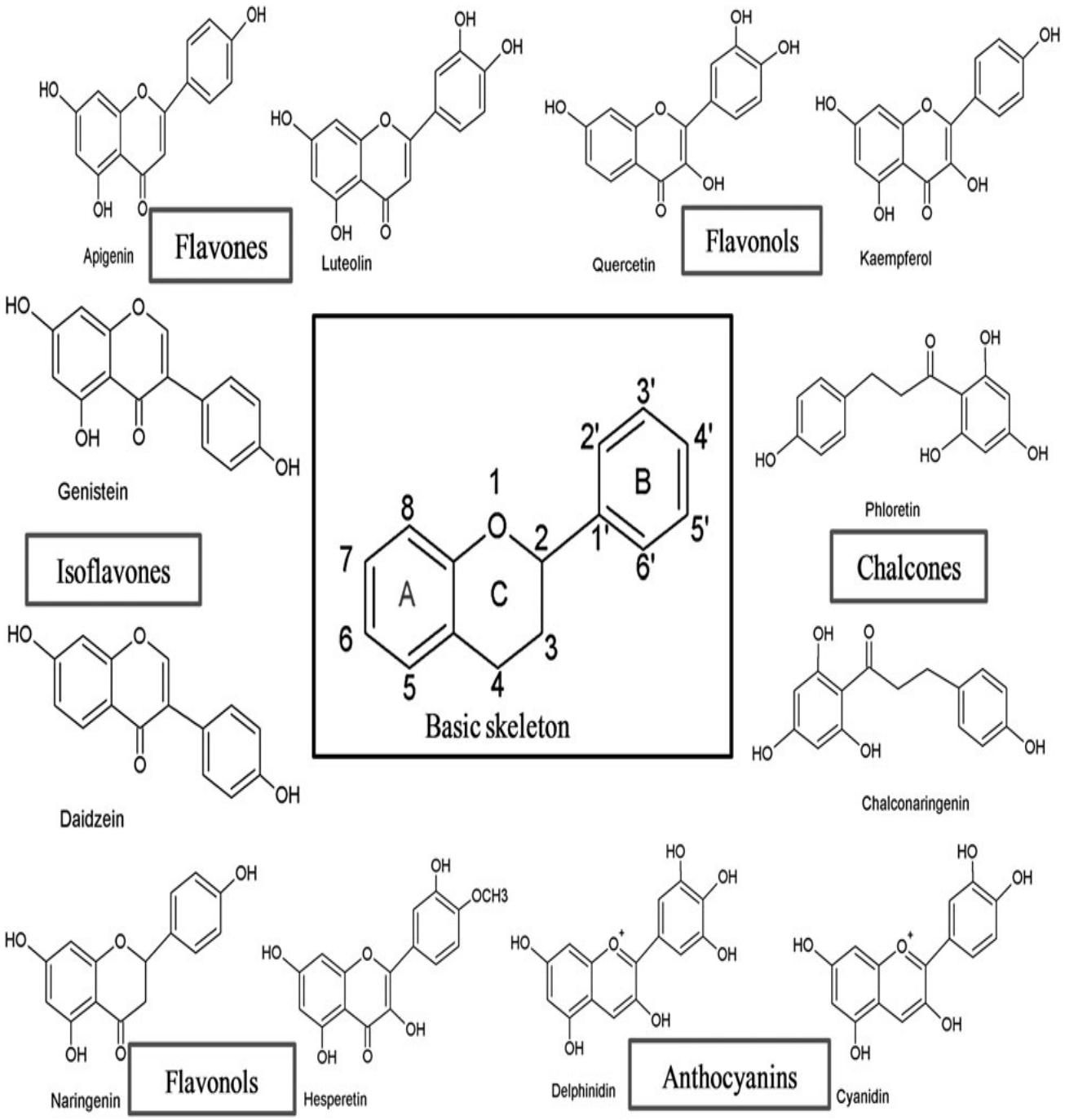


Fig.5. Basic skeleton structure of flavonoids and their classes (Panche *et al.*, 2016).

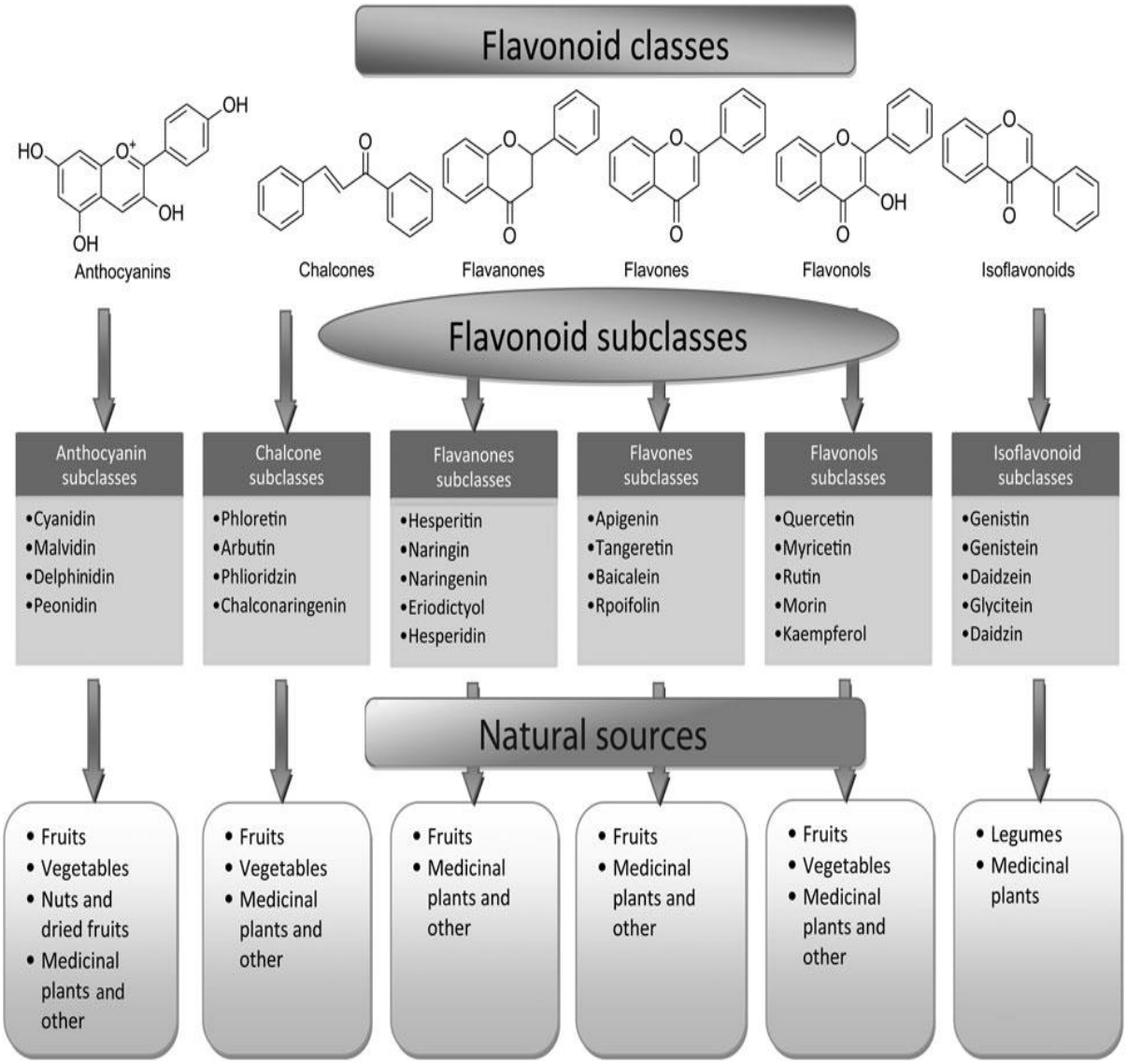


Fig.**6**. Flavonoid classes, subclasses and natural sources (Panche *et al*., 2016).

The flavonoid biosynthetic pathway now is well elucidated, compared to biosynthetic pathways of other secondary products (Dixon and Steele, 1999; Winkel-Shirley, 2001). Flavonoids are synthesized through the phenylpropanoid or acetate-malonate metabolic pathway. Interestingly, flavonoids have similar precursors to those utilized for lignin biosynthesis but exhibit a number of basal structures that result in generation of diverse structures including flavones, flavonols, flavan-3-ols, flavanones, isoflavanones, isoflavans, and pterocarpans (Fig.7).Substitution by glycosylation, malonylation, methylation, hydroxylation, acylation, prenylation, or polymerization leads to diversity in this family and has important impact upon function, solubility, and degradation (Dixon and Steele, 1999; Winkel-Shirley, 2001; Zhang *et al.*, 2009).

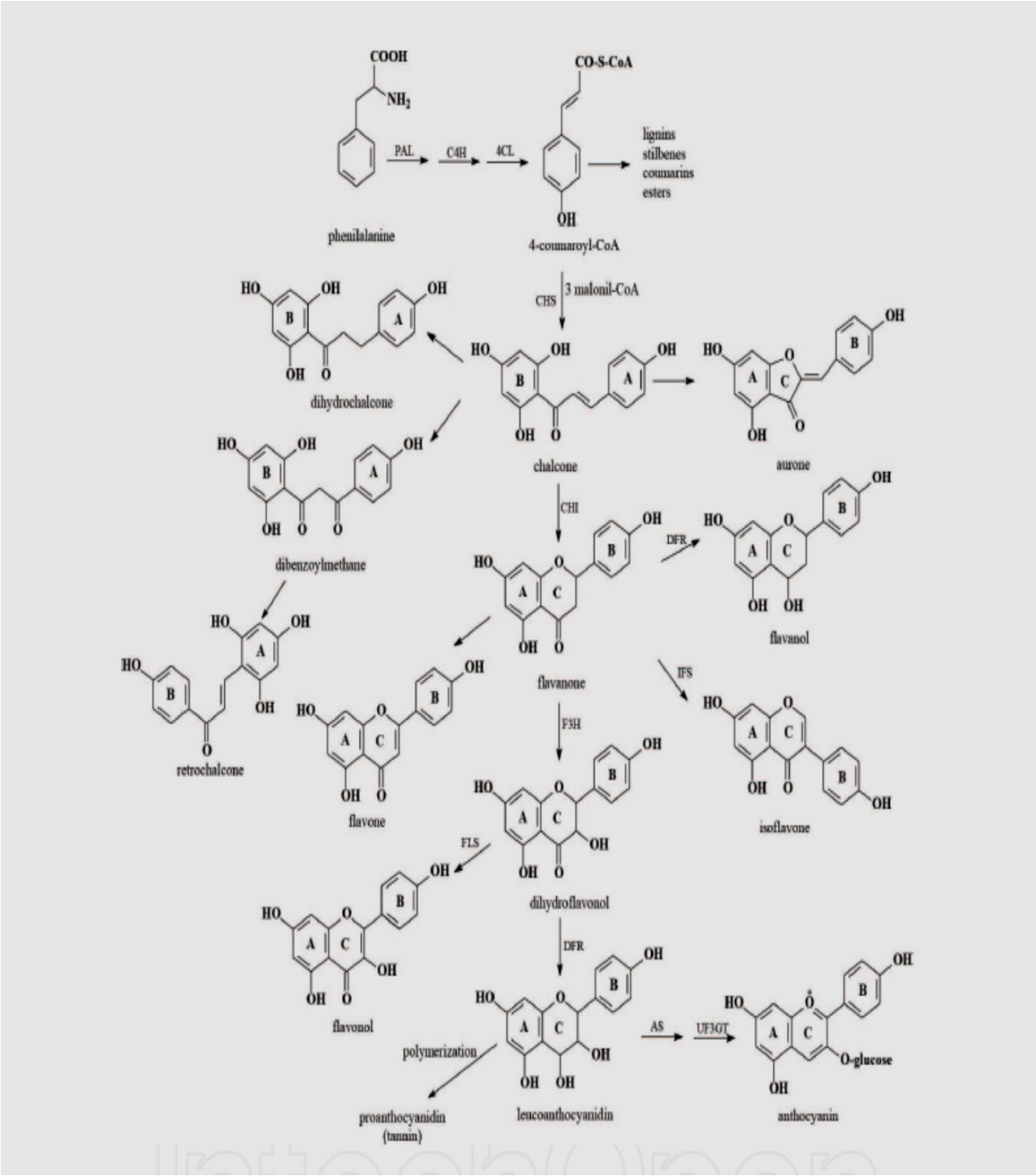


Fig.7. Diagram of the flavonoid biosynthetic pathway. Key enzymes catalyzing some reactions: PAL, phenylalanine amonialyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumaroyl-coenzyme A ligase; CHS, chalcone synthase; CHI, chalcone flavanone synthase; F3H, flavanone 3β-hydroxylase; DFR, dihydroflavonol 4-reductase; FLS, flavonol synthase; IFS, isoflavonoid synthase; AS, anthocianin synthase and UF3GT, UDP glucose: flavonoid 3-O-glucosyltransferase (Erica *et al.*, 2017).

I.4.2.2.1. Generalities on Apiginin

Apigenin (4′,5,7-trihydroxyflavone) (Fig.8) found in many plants, is a natural product belonging to the flavone class that is the aglycone of several naturally occurring glycosides. It is a yellow crystalline solid that has been used to dye wool. Apigenin is found in many fruits and vegetables, but parsley, celery, celeriac, and chamomile tea are the most common sources (Emily, 2013). Apigenin is particularly abundant in the flowers of chamomile plants, constituting 68% of total flavonoids (Venigalla *et al.*, 2015).

Apigenin is biosynthetically derived from the general phenylpropanoid pathway and the flavone synthesis pathway (Forkmann , 1991). The phenylpropanoid pathway starts from the aromatic amino acids L-phenylalanine or L-tyrosine, both products of the Shikimate pathway (Herrmann, 1995). When starting from L-phenylalanine, first the amino acid is non-oxidatively deaminated by phenylalanine ammonia lyase (PAL) to make cinnamate, followed by oxidation at the *para* position by cinnamate 4-hydroxylase (C4H) to produce *p*-coumarate. As L-tyrosine is already oxidized at the *para* position, it skips this oxidation and is simply deaminated by tyrosine ammonia lyase (TAL) to arrive at *p*-coumarat (Lee *et al.*, 2015). To complete the general phenylpropanoid pathway, 4-coumarate CoA ligase (4CL) substitutes coenzyme A (CoA) at the carboxy group of *p*-coumarate. Entering the flavone synthesis pathway, the type III polyketide synthase enzyme chalcone synthase (CHS) uses consecutive condensations of three equivalents of malonyl CoA followed by aromatization to convert *p*-coumaroyl-CoA to chalcone (Austin and Noel, 2003). Chalcone isomerase (CHI) then isomerizes the product to close the pyrone ring to make naringenin. Finally, a flavanone synthase (FNS) enzyme oxidizes naringenin to apigenin (Martens *et al.*, 2001). Two types of FNS have previously been described; FNS I, a soluble enzyme that uses 2-oxogluturate, Fe^{2+}, and ascorbate as cofactors and FNS II, a membrane bound, NADPH dependent cytochrome p450 monooxygenase (Leonard *et al.*, 2005). The naturally occurring glycosides formed by the combination of apigenin with sugars include:

- Apiin (apigenin 7-*O*-apioglucoside), isolated from parsley (Meyer *et al.*, 2006) and celery
- Apigetrin (apigenin 7-glucoside), found in dandelion coffee
- Vitexin (apigenin 8-*C*-glucoside)
- Isovitexin (apigenin 6-*C*-glucoside)
- Rhoifolin (apigenin 7-*O*-neohesperidoside)
- Schaftoside (apigenin 6-*C*-glucoside 8-*C*-arabinoside)

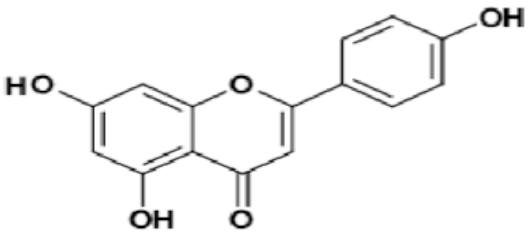


Fig.8. Apigenin (4′,5,7-trihydroxyflavone) (Kamila, 2016)

I.4.2.2.1.1. Roles of Apiginin

Apigenin may also stimulate adult neurogenesis, with at least one study claiming that apigenin stimulates adult neurogenesis *in vivo* and *in vitro*, by promoting neuronal differentiation and may be useful for stimulating adult neurogenesis and for the treatment of neurological diseases, disorders and injuries, by stimulating the generation of neuronal cells in the adult brain. While potentially promising, the study used rats and its effects have yet to be demonstrated in humans (Taupin, 2009). Apigenin readily crosses the blood-brain barrier and has not demonstrated toxicity at high doses. It could thus prevent amyloid beta deposition and tau phosphorylation due to neuroinflammation, which are associated with Alzheimer's disease (Venigalla *et al.,* 2015).Through effects on cell signaling, inflammation, cell cycle, and protease production, apigenin has demonstrated effectiveness against a wide range of cancer types, while not showing toxicity to normal cells (Shukla and Gupta , 2010; Srivastava and Gupta, 2007). Apigenin is able to block the phosphorylation of certain proteins in pathways that, in the case of a cancer, are over expressed like NF-κB, PI3K, etc...(Yan *et al.,* 2017). These pathways can induce proliferation, migration and invasion if not regulated. In addition, several studies have demonstrated that the anticarcinogenic properties of apigenin occur through regulation of cellular response to oxidative stress and DNA damage, suppression of inflammation and angiogenesis, retardation of cell proliferation, and induction of autophagy and apoptosis. One of the most well-recognized mechanisms of apigenin is the capability to promote cell cycle arrest and induction of apoptosis through the p53-related pathway. A further role of apigenin in chemoprevention is the induction of autophagy in several human cancer cell lines (Bokyung *et al.,* 2016).

I.4.2.2.2 Absorption bands of flavonoids

Studies on flavonoids by UV spectroscopy have shown that most flavonoids consist of two major absorption maxima: band II (240–285 nm) which corresponds to the benzoyl system of

the A ring, while band I (300–400 nm) represents the cinnamoyl system of the B ring (Fig. 9)(Fossen and Andersen, 2006; Markham K.R and Mabry, 1975). Functional groups attached to the flavonoid skeleton may cause a shift in absorption. The application of standardized UV (or UV-Vis) spectroscopy has for years been used in analyses of flavonoids (Havsteen, 2002).

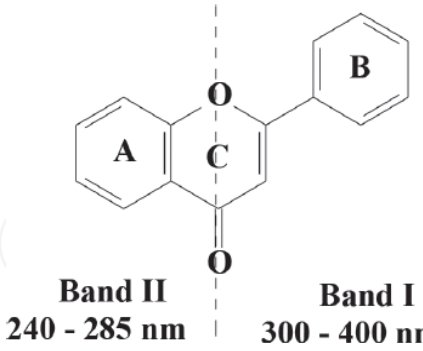


Fig.9. Absorption bands of flavonoids: Band II absorption (originated from A-ring benzoyl system) and Band I (from the B-ring cinnamoyl system). (Erica *et al.*, 2017)

I.5. Generalities on Proteins and oxidative enzymes

I.5.1. Definition

Proteins are macromolecules made up mainly of the elements carbon, hydrogen, oxygen and nitrogen. Sulphur and phosphorus are usually present in some proteins (Green *et al.*, 2002). Proteins are composed of amino acids linked together into polypeptides by peptide bonds. There are 20 proteinogenic amino acids in nature, and they all have different properties, depending on their side chain. In Table.6 the amino acids are listed with the full name, 3-letter code, 1-letter code, chemical formula of the side chain and some of the chemical features. The physical, chemical and biological properties of proteins are determined by the amino acids and their internal order. The amino acid sequence of a polypeptide is called the primary structure (Fig. 10A.). Native proteins are folded into a three dimensional structure, which is crucial for the protein's biological function. To reach this structure, the polypeptide will first adapt certain secondary structures depending on the order of the amino acids. Periodical amino acid sequences will give α-helices and β-pleated sheets (Fig.10B.), while more irregular sequences form loops and other disordered structures. The folding of a polypeptide is finished when the tertiary structure is reached. The tertiary structure is the overall assembly of the peptide chain and is mainly stabilized by internal disulfide bonds, hydrogen bonds, hydrophobic and ionic interactions (Fig. 10C.). Usually the bioactive protein

has a quaternary structure, where several polypeptides, called subunits, form a complex (Fig. 10D.) stabilized by hydrogen bonds, hydrophobic and electrostatic interactions (Berg *et al.*, 2005).

Table 6: The 20 natural proteinogenic amino acids, and the chemical formulas of the side chains are presented, as well as some chemical properties: The class of the side chain, the charge at pH 7.4, the isoelectric point (pI), and the normalized hydrophobicity of the amino acids. The abundance of the amino acids in proteins from all kingdoms is given as % of all amino acids in the proteome-pI database (Kozlowski, 2017). The essential amino acids for humans are marked with *. Based on NCBI Amino Acid Explorer (NCBI) and †(Kozlowski, 2017).

Amino acid	**Side chain**	**Side chain class**	**Charge at pH 7.4**	**pI**	**Hydro-phobicity**	**Abundance %**
Alanine Ala or A	CH_3	aliphatic		6.01	0.806	8.76
Arginine* Arg or R	$(CH_2)_3NH$-$C(NH)NH_2$	basic	positive	10.76	0.000	5.78
Asparagine Asn or N	CH_2CONH_2	amide	polar	5.41	0.448	3.93
Aspartic acid Asp or D	CH_2COOH	acid	negative	2.85	0.417	5.49
Cysteine Cys or C	CH_2SH	S containing	polar	5.05	0.721	1.38
Glutamic acid Glu or E	CH_2CH_2COOH	acid	negative	3.15	0.458	6.32
Glutamine Glu or Q	$CH_2CH_2CONH_2$	amide	polar	5.65	0.430	3.9
Glycine Gly or G	H	aliphatic		6.06	0.770	7.03
Histidine* His or H	CH_2-$C_3H_3N_2$	Basic aromatic	positive	7.60	0.548	2.26
Isoleucine* Ile or I	$CH(CH_3)CH_2CH_3$	aliphatic		6.05	1.000	5.49
leucine* Leu or L	$CH_2CH(CH_3)_2$	aliphatic		6.01	0.918	9.68
lysine* Lys or K	$(CH2)_4NH_2$	aliphatic	positive	9.60	0.263	5.19
Methionine* Met or M	$CH_2CH_2SCH_3$	S containing		5.74	0.811	2.32
Phenylalanine* Phe or F	$CH_2C_6H_5$	aromatic		5.41	0.951	3.87
Proline Pro or P	$CH_2CH_2CH_2$	cyclic		6.30	0.678	5.02
Serine Ser or S	CH_2OH	OH	polar	5.68	0.601	7.14
Threonine* Thr or T	$CH(OH)CH_3$	OH	polar	5.60	0.634	5.53
Tryptophan* Trp or W	$CH_2C_8H_6N$	aromatic		5.89	0.854	1.25
Tyrosine Tyr or Y	CH_2-C_6H_4OH	aromatic	polar	5.64	0.714	2.9
Valine* Val or V	$CH(CH_3)_2$	aliphatic		6.00	0.923	6.73

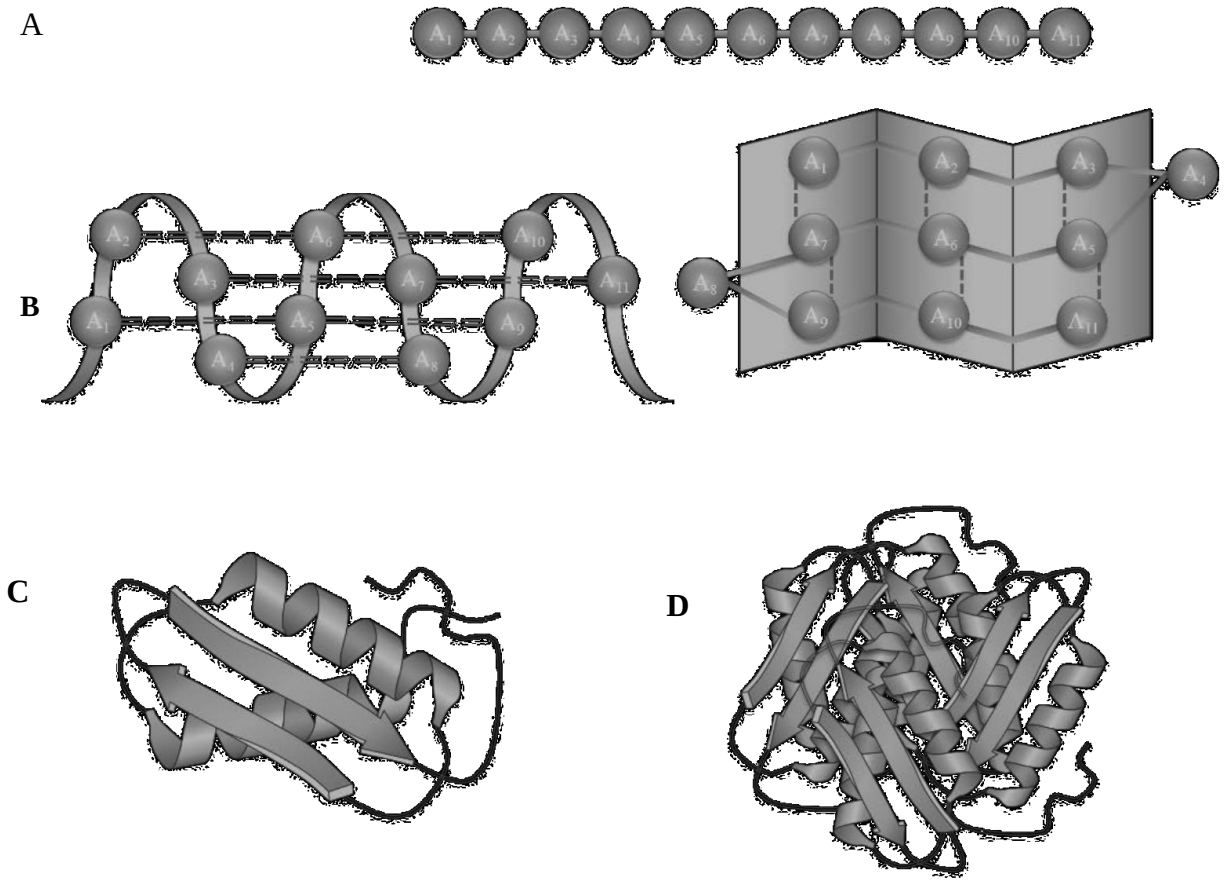


Fig.10. The levels of protein structure. A: Primary structure: Amino acids in sequence. B: Secondary structure: The chain of amino acids folds into α-helices and β-sheets. C: Tertiary structure: The partly folded amino acid chain adapts a three dimensional structure. D: Quaternary structure: Several chains, called subunits, form a complex (Nynäs, 2018).

I.5.2 Role of proteins in plants

Proteins found in plants have diverse functions which include catalysis, structural, defence, stress response and storage. Enzymes are proteins that direct and accelerate biochemical reactions**:** Some plant proteins provide structural support and have significant mechanical strength; A variety of proteins have defence functions. Some of these molecules can play a physiological role in plant defences, as is the case with albumins from radish seeds that have shown an interesting inhibition activity against fungi and bacteria (Agizzio *et al*.,2003); Certain proteins serve as a reservoir of essential nutrients. Plant storage proteins (albumins, globulins, prolamins and glutelins) are associated with specific plant components, such as seeds, nuts, and kernels; stem parenchyma of trees; grains and legumes; and some roots and tubers. These organs are responsible for protein synthesis and storage, presenting high protein content. The storage proteins make up an excellent source for amino acids which

can be mobilized and utilized for maintenance, defence, and growth of plants, as well as in the embryonic and developmental stages (Muntz, 1998).

The capacity of plants to survive a variety of abiotic and biotic stresses is mediated by certain plant proteins for example, the production of heat shock proteins (hsp) in excessively high temperatures that will promote the correct refolding of damaged proteins. Peroxidases are plant proteins that intervene in the biosynthesis of lignin and resistance to stress, thereby facilitating acclimatization (Shivakumar *et al.,* 2002).

I.5.3. Leaf proteins

Green leaves have long been considered as a possible protein source for sustainable food and feed production. Proteins in green leaves can be divided into a white and green protein fraction. The white protein fraction is mainly RuBisCO, or ribulose-1,5-bisfosfat-caroboxylase/oxygenase (E.C 4.1.1.39), which has been called the most abundant protein in the world, while the green fraction consists of chlorophyll related proteins (Nynäs, 2018). Ribulose-1,5-bisphosphate carboxylase/oxygenase catalyzes the fixation of CO_2, a central reaction of the Calvin–Benson cycle. This key photosynthetic enzyme constitutes the main entry point of CO_2 into the biosphere. Its abundance and richness in sulfur-containing amino acids make it a prime source of N and S during nutrient starvation, when photosynthesis is downregulated and a high RuBisCO level is no longer needed (Wojciech *et al.,* 2019).

I.5.4. Tuber crude proteins

Crude proteins are all the proteins that can be found in a plant or sample. Tubers contain proteins which vary from 1 – 2 % dry weight in cassava up to 10 % in yam bean (*Pachyrhizus spp*) (Shewry, 2003). These proteins contents are usually lower than those of grains, whose proteins contents range from about 10 % in some cereals to about 40 % in soya beans (Shewry, 2003). In *Dioscorea* species, proteins account for 1 -3 % of fresh weight and over 80 % of these proteins represents storage proteins.

I.5.5. Tuber storage proteins

Storage proteins can be defined as proteins whose major role is to act as stores for nitrogen, sulphur and carbon. They may enable the plant to survive periods of adverse conditions or between growing seasons and also may provide nutrients to support the growth

of new plants like seedlings from seeds or shoots from tubers. According to Shewry and Casey (1999), seeds contain four defined categories of storage proteins due to their difference in solubility in chemical solutions. These are Prolamine, albumins, 7S globulins and 11S globulin. Prolamins and glutelins are grouped together as prolamins and some researchers simplify this into two super-families: prolamins (albumins, prolamins and glutelins) and globulins (Guillaume, 2007). The majority of these proteins demonstrated not to have any biological activity. However, some albumins have been found to exhibit antifungal properties and to inhibit serine proteinases (Genov *et al.*, 1997). These activities are seen as secondary role. Plant storage proteins account for more than half the crude proteins and are constituted of a mixture, in variable proportion of diverse protein categories (Nasri and Triki, 2007).

Tubers also possess storage proteins and these proteins vary from tuber to tuber. In potato, patatin exist as storage protein, sporamin in sweet potato, dioscorin in yams and globulin1 and globulin2 in cocoyams (Shewry, 2003). All these storage proteins play defence roles through different means. Sporamin and dioscorin exhibit antioxidant and radical scavenging activities. Patatin exhibits activities like esterase and acylhydrolase, while the globulins of cocoyams are trypsin inhibitors (G2) and mannose binding lectin. Storage proteins are not structurally related to other storage proteins such as patatin from potato (*Solanum tuberosum*) and sporamin from sweet potato (*Ipomoea batatas*) (Gaidamashvili *et al.*, 2004**).**

1.5.6. Peroxidases

Peroxidases are enzymes that contain heme as the cofactor (hemoproteins) that exist in many higher plants. They are located in cell walls, cytoplasmic inclusions (lysosomes, mitochondria and chloroplasts) and also in membranes. According to their bonds with the cell elements, peroxidases can be classified into three (3) groups:

- Soluble peroxidases that are extracted after grinding the tissues in a buffer solution of low ionic strength;
- Peroxidases ionically bound to cell walls and membranes that are extracted with a buffer of high ionic strength; and
- Covalently bound peroxidases. They are liberated through enzymatic digestion of the cell wall.

These enzymes have a variety of functions as evidenced by the presence of several isoenzymes in plant cell compartments. They intervene in the growth, development and the defence systems of the plant (Karthikeyan et al., 2005; Baaziz *et al*., 2006). In the process of growth, peroxidases control the levels of endogenous auxin through their action on 3-indol acetic acid (Kim *et al*., 1996). Also, peroxidases breakdown auxins, by so doing, they participate in controlling apical growth of plants (Gaspar *et al*., 1996). class III plant peroxidases (EC 1.11.1.7; hydrogen donor: H_2O_2 oxidoreductase, Peroxidases) are involved in the control of cell elongation, polymerization of extensin, cross-linkage of cell wall polysaccharides, lignin biosynthesis and the suberization processes (Almagro *et al.,* 2009).

At the level of cell walls, peroxidases also participate in the oxidation of a range of phenolic compounds generated from cinnamic acid to toxic products for pathogens (Kawano, 2003) and the transformation of aromatic amino acids to quinines and phytolexins that have fungal and bactericidal properties. Their action on cell wall rigidity permits these cell walls to be less sensitive lytic enzymes of parasites. In general, in the case of wound, infection or all other unfavourable conditions, new isoperoxidases appear (Djocgoue, 1998; Mas Athira and Khong, 2019).

1.5.7. Polyphenol oxidases

Polyphenol oxidases (PPOs) are a group of copper containing enzymes that catalyze the o-hydroxylation of monophenols to o-diphenols (tyrosinase activity, Enzyme Commission (1.14.18.1) as well as the oxidation of o-diphenols to quinones (catecholase activity, Enzyme Commission (1.10.3.2) in the presence of oxygen. (Soha *et al.,* 2014). These enzymes are broadly distributed among animals, fungi, and plants, though many plant PPOs appear to lack tyrosinase activity (Steffens *et al.,* 1994). The study of PPOs in plants has focused primarily on their role in the process of postharvest browning, whereby cut or damaged plant tissues turn brown due to the polymerization of PPO generated quinones, generating phytomelanins (Mesquita and Queiroz, 2013). Found in high concentrations in potato tuber peel and 1-2 mm of the outer cortex tissue, PPO is used in the potato as a defense against insect predation, leading to enzymatic browning from tissue damage. Damage in the skin tissue of potato tuber causes a disruption of cell compartmentation, resulting in browning. The brown or black pigments are produced from the reaction of PPO quinone products with amino acid groups in the tuber.(*Thygesen et al.,* 1995) In potatoes, PPO genes are not only expressed in potato tubers, but also in leaves, petioles, flowers and roots (*Thygesen et al.*, 1995) Classically,

PPOs and their potential phenolic substrates have been considered to be physically separated from one another in intact plant cells, with most PPOs targeted to the chloroplasts, while phenolic compounds accumulate primarily in the vacuole and cell wall (Vaughn *et al.,* 1988; Steffens *et al.,* 1994). Thus, PPO activity in vivo has typically been associated with senescing, wounded, or damaged plant tissues in which cellular compartmentalization is lost (Steffens *et al.,* 1994).

1.5.8. β-1,3-Glucanases

Karthikeyan *et al.,* (2006) investigated the effect of soil application of biocontrol agents (*Pseudomonas fluorescens*, *Trichoderma viride* and *T. harzianum)* in combination with chitin on induction of phenolics and defense enzymes in coconut roots infected with *Ganoderma lucidum*, the causal agent of *Ganoderma* disease. Soil application of these biocontrol formulations in combination with chitin induced a significant increase in the activities of peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), chitinase and β-1,3-glucanase in the *G. lucidum* infected palms. β-1,3-Glucanases are enzymes which regulate the degradation of callose or (1,3)-β-D-glucan which is a main component in the cell walls of higher plants. (Moravčíková *et al.,* 2016). β-1,3-Glucanases are abundant in plants and have been characterized from a wide range of species. They play key roles in cell division, trafficking of materials through plasmodesmata, in withstanding abiotic stresses and are involved in flower formation through to seed maturation. They also defend plants against fungal pathogens either alone or in association with chitinases and other antifungal proteins. They are grouped in the PR-2 family of pathogenesis-related (PR) proteins. Use of β -1,3-glucanase genes as transgenes in combination with other antifungal genes is a plausible strategy to develop durable resistance in crop plants against fungal pathogens. These genes, sourced from alfalfa, barley, soybean, tobacco, and wheat have been co-expressed along with other antifungal proteins, such as chitinases, peroxidases, thaumatin-like proteins and a-1-purothionin, in various crop plants.

with promising results (Vaiyapuri *et al.,* 2012). Innoculation of cocoa plants with heat treated oyster shell powder enhances accumulation of β -1,3-glucanase in leaves and improving tolerance to *Phytophthora megakarya* (Martial *et al.,* 2019)

CHAPTER II: METHODOLOGY

II.1. Experimental site

A field trial was conducted to study the performance of *Xanthosoma sagittifolium* mintuber seeds as influenced by poultry manure and NPK fertilizer during the 2017 cropping season on an experimental farm at Ngog Bibega, Mbankomo Sub-division, (Outskirsts of Yaoundé) Centre region, Cameroon, located at latitude 3°49'52.5"N and longitude 11°27'15.79"E and 714 m above sea level. This area is characterized as a humid rainforest zone with a clay loam soil located in the bimodal humid forest agro-ecological zone in cameroon. The total annual rainfalls for 2017 was 1902.8 mm while the total rainfalls during the period of experimentation (April to December) for 2017 was 1775.6 mm (Fig. 11).

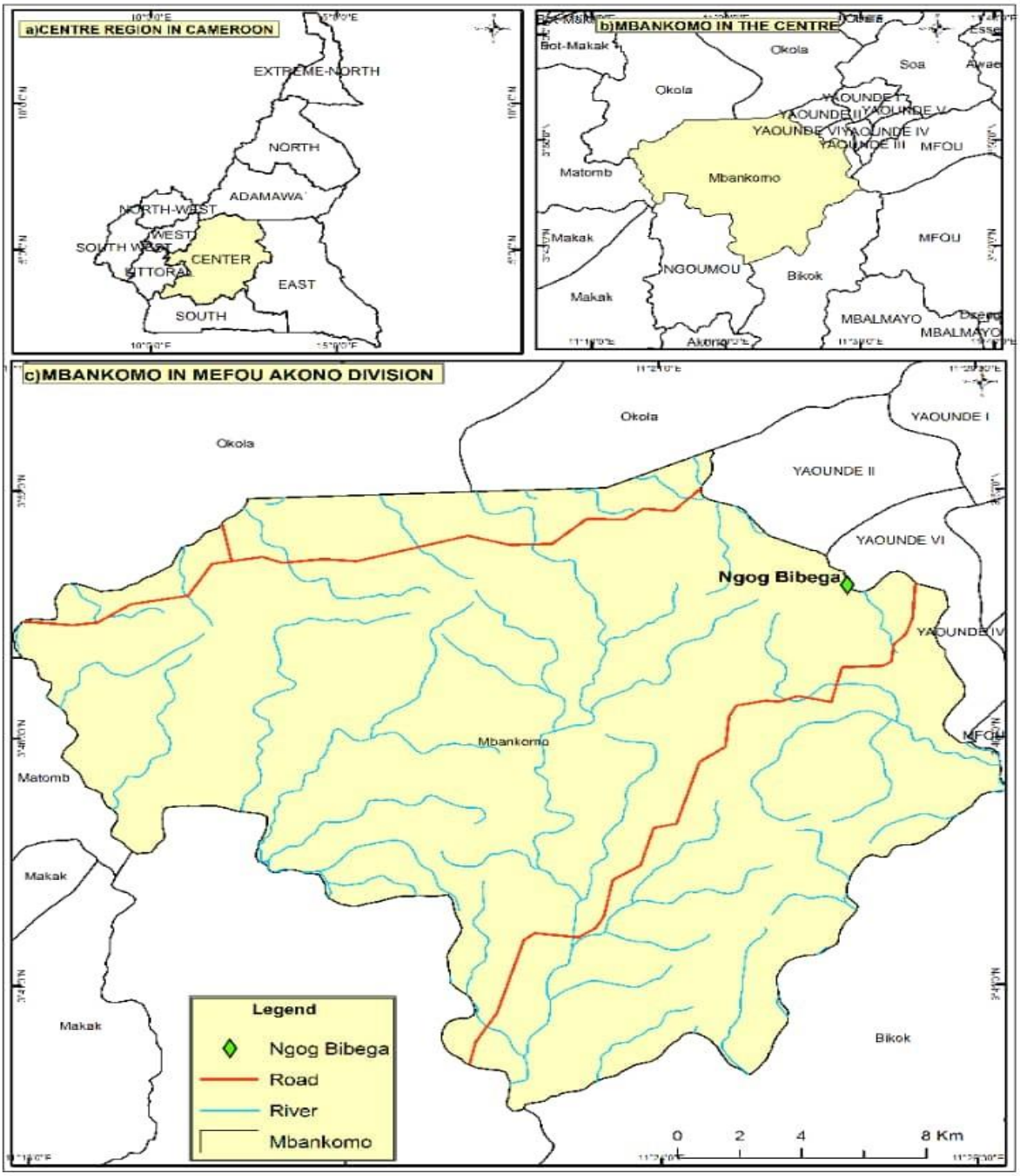


Fig.11. Geographical location of experimental farm (Ngog Bibega).

II.2. Materials

II.2.1. Plant material

The planting material consisted of white and red cultivars of *X. sagittifolium* minituber seeds of mean weight 38g produced from acclimatized vitroplants under the shed in the plant physiology Laboratory of the Higher Teacher Training College (HTTC), University of Yaounde I. Yaounde, Cameroon according to the protocol of Djeuani *et al*. (2014, 2017).

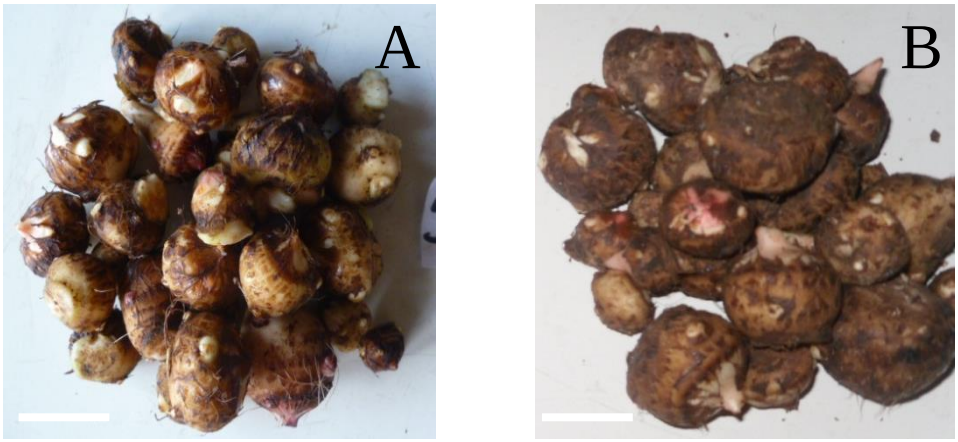


Fig.12. Aspect of X. sagittifolium minitubers used as seeds: white cultivar (A) and red cultivar (B). Scale = 1cm (Photo Gwan, 2017)

II.2.2. Fertilizers

Poultry manure was obtained from “Henri et Frères” Poultry farm Yaoundé Cameroon and was stored for one month before application. Poultry manure samples were dried in a forced-air oven at 60°C and ground to 0.5 mm for chemical analysis (Table 7). The NPK fertilizer (20:10:10) was obtained from the fertilizer unit of the Centre Regional delegation of Agriculture and Rural Development, Yaoundé, Cameroon.

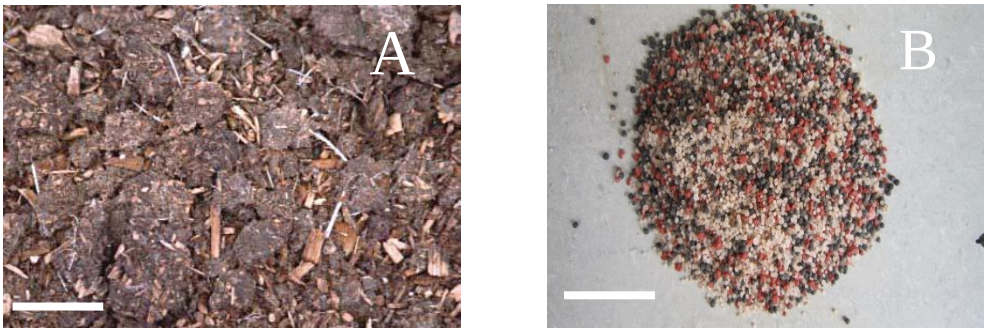


Fig. 1. Aspect of fertilizer used: Poultry maure (A) and NPK (20:10:10) fertilizer (B). Scale=1cm (Photo Gwan, 2017).

Table 7: Chemical characteristics of the poultry manure applied.

Parameter	value
pH (H_2O)	6.9
pH (KCl)	6.8
Organic matter (%)	41.3
Organic carbon (%)	32.3
Total nitrogen (%)	2.11
Available phosphorus (%)	0.89
Potassium (%)	0.99
Calcium (%)	1.06
Magnesium (%)	0.36
C/N	6.9

Source : Gwan, (2019).

II.3. Methods

II.3.1 Experimental design and treatments

The experiment was a 4×2 factorial arrangement in a randomized complete block design and replicated three times for each variety. The site was ploughed, ridged and marked out into two main blocks, one for each cultivar. Each of these main blocks was further subdivided into 3 sub blocks which represent the three replicates. Each sub block was divided into five experimental plots, thus a total of fifteen plots were used for each cultivar. Each gross plot measured 4m×3m (12 m^2) with a net plot of 2m×2m. Soil samples were collected with soil auger at a depth of 0 to 20 cm from different locations of the site and bulked into

composite sample. The treatments comprised three rates each of application of Poultry Manure (0, 20, and 30 $t.ha^{-1}$) and NPK fertilizer (0, 120, and 150 $kg.ha^{-1}$). A total of fifteen treatment combinations and three replications were used. The Poultry manure was incorporated into the soils on the experimental plots in a single application based on the treatment combinations, at 2 weeks before planting to ease decomposition, while the NPK fertilizer was applied to the cocoyam stands according to treatment allocation at 4 weeks after planting (WAP) using the ring placement method. Each minituber was planted per hole at a depth of 15 cm and at a spacing of 0.5 m x 1.0 m resulting to twenty-five plants per plot and a total of 375 plants per cultivar. All plots were kept weed free by manual weeding after every 2 weeks.

II.3.2. Soil analysis

Soil samples were collected with soil auger at a depth of 0 to 20 cm from different locations of the site and bulked into composite sample. The composite soil sample was air dried, passed through 2 mm sieve, and then analyzed for its physicochemical properties before planting and after harvest.

II.3.2.2. Determination of physical properties of soils

Particle size distribution was performed to determine the soil textural class using the modified hydrometer method of Gee and Bauder. (1986); Kettler *et al.* (2001) as follows:

- Soil-particle dispersion was accomplished by adding Hexamephosphate (HMP), at an aqueous concentration of 0.5% by weight and shaking the soil sample (2 mm) for 16 hours on a reciprocating shaker at 120 reciprocations per minute in a container with a 3:1 HMP (90 ml) to soil (30 g) ratio.

- After dispersion, the soil slurry was sieved through nested standard 0.5-mm mesh (no. 35) and 0.053-mm mesh (no. 270) sieves to separate sand particles and Particulate organic matter (POM) while the solution containing clay and silt was collected into a 1l beaker.

- The collected sand particles (0.053 mm) were dried at 55 ^{o}C to constant weight, then subjected to 450 ^{o}C for 4 h to measure POM by loss on ignition. The sand percentage

was based on its fraction of the original sample mass and was calculated using the mass of sand at 55 ^{o}C.

- The solution in the 1l beaker was then stirred and 50 ml was transferred into a centrifuge tube and allowed to stand for 2hours to allow silt particles to sediment.

- After sedimentation the suspended solution containing clay was decanted into a drying pan while the silt particles were rinsed and put into another drying pan and both were dried to constant weight at 105 ^{o}C.

- The relative proportion of silt in the dried subsample was calculated as:

    - **Total subsample mass = oven dry silt mass + oven dry clay mass**

    - **Subsample silt =** $\frac{\textbf{oven dry silt mass}}{\textbf{total subsample mass}}$

- This value was then used to calculate the percentages of silt and clay in the original sample:

    - **Sand %** $= \frac{\textbf{Oven dry sand mass}}{\textbf{Original sample mass}} X \frac{100}{1}$

    - **(Silt + Clay) % =100 - Sand%**

    - **Silt% = Subsample silt X (Silt + Clay) %**

    - **Clay% = 100 - (Sand% + Silt%)**

II.3.2.3 Measurement of soil pH.

Soil pH was determined in distilled water and potassium chloride solution using pH meter using the method of Mclean, 1982.

For soil pH in water the following procedure was used:

15g of sieved air dried soil was weighed into each of 2 labeled 50 ml centrifuge tubes

- 30 ml of distlled water was then added into each of the centrifuge tubes (for a soil: water of 1: 2) and covered with lids;

- the tubes were shaken to form a soil slurry;
- the lids were removed and the tubes let to stand for 30 minutes;
- the pH meter was standardized using pH 4 and 7;
- the soil slurry was swirled gently while taking pH measurement.

For soil pH in KCl the following procedure was used:

- 15g of sieved air-dried soil was weighed into each of 2 labeled 50 ml centrifuge tubes;
- 30 ml of 0.01M KCl was then added into each of the centrifuge tubes (for a soil: 0.01M KCl of 1: 2) and covered with lids;
- the tubes were shaken vigourously to form a soil slurry;
- the lids were removed and the tubes let to stand for 30 minutes;
- the pH meter was standardized using pH 4 and 7;
- the soil slurry was swirled gently while taking the pH measurement.

II.3.2.4. Determination of soil nitrogen

Soil nitrogen was determined by Macro-Kjeldahl digestion method (Bremner and Mulvaney, 1982; Kjeldahl,1983) using the following steps:

- 50g of sieved soil sample was weighed and put into a 500 ml Kjeldahl flask;
- 1g $CuSO_4$, 10 g K_2SO_4 and 30 ml Con. H_2SO_4 was added, mixed and allowed to stand for 30min;
- digestion was effected on the Kjeldahl digestion rack with low flame for the first 10 – 30 min until the frothing stops and then gradually more strongly until the sample was completely charred. The heat was gradually raised until the acid reached approximately one third the way up the digestion-flask;

- the content was cooled and diluted to about 100 ml with distilled water and the fluid part was transferred into a 1000 ml distillation flask. The residue was also rinsed and the washings decanted into the distillation flask.

- the flask was fitted with two neck joints. To one neck a dropping funnel was connected for adding 40 % NaOH while the other neck, Kjeldahl trap, was used to trap the NaOH coming with the distillate. The trap was connected to the condenser with a delivery tube which dipped into 50 ml of 0.1 N HCl contained in a conical flask, with two drops of methyl red indicator;

- 125 ml of 40 % NaOH solution was added till the content was alkaline in reaction and heated;

- the ammonia formed was absorbed in standard HCl and when no more ammonia was received the distillation was stopped;

- the excess of the acid was titrated with 0.1N NaOH solution till the pink colour changed to yellow;

- From the titre value the multi equivalence of the acid participating in the process of ammonia absorbing during digestion was calculated.

Calculations:

i. Blank:

- Volume of HCl taken for blank = $\underline{a}$ ml

- Volume of NaOH used = $\underline{b}$ ml

- Volume of HCl consumed by liberated NH_3 present in blank = a – b = $\underline{z}$ ml

ii. Sample:

- Volume of HCl taken for sample = $\underline{v}$ ml

- Volume of NaOH used = $\underline{u}$ ml

- Volume of HCl consumed by liberated NH_3 present in sample = v – u = $\underline{w}$ ml

- Volume of HCl consumed for NH_3 liberated by sample only = w – z = $\underline{y}$ ml
- 1000 l 1N HCl = 1000 ml 1 N NH_3 = 17 g NH_3 = 14 g N
- 1 ml 1N HCl = 1 ml 1 N NH_3 = 0.014 g N
- 1 ml 0.1 N HCl = 1 ml 0.1 N NH_3 = 0.0014 g N

Weight of Nitrogen in 5 g of Sample = y x 0.0014 g N = $\underline{q}$ g N

$$\%\ of\ N\ in\ sample = \frac{qx100}{5} = P\%$$

II.3.2.5. Determination of soil phosphorus (P)

Available soil P was determined by Bray II method (Bray and Kurtz, 1945) with the following procedure:

- 5 g soil was weighed and transferred it to a 100 ml conical flask;
- 50 ml extract solution (containing 0.03 N NH_4F and 0.025 N HCl) was added to the soil;
- the contents were shaken for 5mins and filtered through Whatman No. 42 filter paper;
- a blank was prepared in which all the reagents were added similarly, except the soil;
- Seven 25 ml standard flasks were taken and labelled as sample, blank, 0.2, 0.4, 0.6, 0.8, 1;
- 5 ml of soil extract was pipetted out into one 25ml standard flask which was labeled as sample
- 5 ml of Dickman and Bray's Reagent was transferred into 25 ml standard flask containing the soil extract and 7.5 ml Boric acid added;
- the Standard Phosphorus solution was taken in a clean burette and 1,2,3,4 and 5ml each added in previously labeled 25 ml standard flasks (except in "blank");

- 5 ml of Dickman and Bray's Reagent was transferred into each 25 ml standard flask containing the Standard Phosphorus solution. To each 7.5 ml Boric acid was added;
- a test tube full of distilled water was added through the neck of the flask down to remove the adhering Ammonium Molybdate and the content thoroughly mixed;
- Finally, 1ml $SnCl_2$ (Tin (II) Chloride) working solution was added with immediate mixing and made up to the mark with distilled water once again, mix the solution thoroughly;
- Similarly, a blank was prepared;
- the intensity of blue colour was measured just after 10 minutes at 690 nm;
- a graph between absorbance against the concentration in ppm was plotted to determine the concentration of P in soil samples from the standard curve.

Calculation

$$\textbf{ppm P in soil} = \textbf{ppm P in solution (grom graph)} \textbf{x} \frac{\boldsymbol{Total\ mL\ extracting\ solution\ used}}{\boldsymbol{Weight\ of\ soil}}$$

II.3.2.6. Determination of soil organic carbon

Soil organic carbon was determined by the method of Schumacher (2002) as follows:

- 1 g of soil was put in a 500 ml conical flask;
- 10 ml of 1N $K_2Cr_2O_7$ (Potassium dichromate) solution was added and shaken to mix it;
- 20 ml Con. H_2SO_4 (Sulphuric acid) was added and the flask swirled 2 times;
- the flask was allowed to stand for 30 minutes on an asbestos sheet for the reaction to be completed;
- 200 ml of distilled water was poured into the flask to dilute the suspension;
- 10 ml of 85% H_3PO_4 (Phosphoric acid) and 1 ml of Diphenylamine indicator was added and the solution was back titrated with 0.5 N $(NH4)_2Fe(SO4)_2$ (Ferrous Ammonium Sulphate);
- till the colour flashed from violet through blue to bright green;

- the volume of Ferrous Ammonium Sulphate was recorded;
- a blank titration (without soil) was carried out in a similar manner.

Calculation:

% of Organic Carbon in Soil (R) is,

$$R = \frac{(V_1 - V_2) X\ NX\ 0.003\ X\ 100}{W} X\ C$$

Where:

W = Weight of Sample

V_1 =Blank Titre value

V_2 =Titre value of the Sample

N =Normality of $K_2Cr_2O_7$ (Here it is 1N)

C = Correction Factor (1.334, 1.724)

II.3.2.7. Determination of exchangeable cations (Ca^{++}, Mg^{++}, K^+, Na^+) and calculation of cation exchange capacities (CEC).

Determnation of exchangeable cations (Ca^{++}, Mg^{++}, $K^{+,}$ Na^+) and calculation of cation exchange capacities (CEC) was carried out according to the methods of Warncke and Brown (1982) and Thomas (1982) with the following procedure:

- 1.5 g of soil was placed into a 50ml Erlenmeyer flask;
- 15 ml of extracting solution (1 N NH_4OH, pH 7.0) was added by constant suction pipette.
- the suspension was shaken on an oscillating shaker for 15 minutes;
- the solution was filtered through Whatman No. 2 filter paper into 15ml funnel tubes. Acid washed filter papers were used for Na extrations;

Ca, Mg, K and Na in the filtered extract were determined via atomic absorption spectrophotometry, using a bulk standard containing 40 ppm of Ca / Mg (run simultaneously); 15 ppm of K; or 15 ppm of Na respectively; which was diluted by the atomic absorption to make Standards. Ca and Mg determinations were made using a nitrous oxide–acetylene flame.

Calculations of cation exchange capacity (CEC)

Results were reported as ppm of exchangeable Ca, Mg, K and Na in soil. The results were used to estimate cation exchange capacity (CEC), by converting ppm of each cation to meq/100g of soil, then summing the values for the four cations:

$$\textbf{Estimated CEC} = \frac{[\textbf{Ca (ppm)}}{\textbf{200}} + \frac{\textbf{Mg (ppm)}}{\textbf{122}} + \frac{\textbf{K (ppm)}]}{\textbf{339}} + \frac{\textbf{X (soil + tare)} - \textbf{tare}*}{\textbf{4.25}}$$

$$\frac{[(\textbf{soil + tare}) - \textbf{tare}*\ ]}{\textbf{4.25}} = \textbf{Soil density}$$

II.3. Evaluation of morphological parameters

II.3.3.1. Growth parameters

Five cocoyam plants were randomly selected from each of the net plots, tagged and then used for the determination of average plant height (cm), average number of leaves, average leaf area and average root number at 1, 2, 3,4,5,6,7, and 8 months after planting (MAP).

- A metre tape was used to measure the height of tagged plants and the mean height calculated;
- The number of leaves of tagged plants were counted manually and the average number determined;
- The length (cm) and width (cm) of leaves of the tagged plants were measured with a metre tape. The leaf area was determined using the formula of Biradar (1978) as:
    - Leaf Area of Cocoyam = 0.917 (LW). Where L and W are length and width of the cocoyam leaf.

II.3.3.2 Yield parameters

Yield parameters like number of tubers per plant, length (cm) and girth (cm) of tubers and tuber weight (g) were assessed in 5 plants per treatment after 9 months at physiological maturity as follows:

- The number of tubers per tagged plant were counted manually at harvest and average number calculated for each treatment;

- A metre tape was used to measure the length and girth of the harvested tubers and the mean lengths and girths calculated for tagged plants of each treatment;

- The tuber weight was determined with a 0.01 electronic balance and mean weight calculated for tagged plants of each treatment (Gwan, 2019).

II.3.4. Determination of macromineral nutrients in cocoyam tubers

The following procedure was used to determine Ca, Mg, K, Na and P contents.

- The cocoyam tuber samples were dried in an oven at 60^{o}C for 2 hours and finely ground;

- 500 mg of finely ground sample was digested in 2 mL concentrated HNO_3;

- The mixture was diluted to 50 mL;

- Instrument calibration standards were prepared from certified standards and both standards and samples read on the flame Atomic Absorption Spectrophotometer (AAS) (J. Benton Jones and Vernon W. Case). Ca was read at 422.7 nm, Mg at 285.2 nm, K at 766.5 nm and Na 589 nm;

- P was extracted as above that is digested in concentrated HNO_3 and analyzed using Murphy Riley reagent and read colorimetrically using a UV-VIS spectrophotometer. P was read at 860 nm.

Total N was determined from a wet acid digest (Buondonno *et al.,* 1995) by colorimetric analysis using a UV-VIS spectrophotometer (Anderson and Ingram, 1993). N was read at 655 nm.

II.3.5. Biochemical analysis

From the analyses of the agro morphological parameters the treatments with the most significant means of traits, together with the control treatments were used for biochemical analyses. Biochemical analyses consisted of extraction and assay of total soluble proteins, peroxidases, β-1,3-glucanases, polyphenoloxidases, total phenol content and flavonoid content in the leaves every month for a period of 6 months.

II.3.5.1. Protein extraction and assay

Proteins were extracted according to the modified method of Pirovani (2008). 1g of leaves were ground in chilled mortar with 5 ml of Tris- Maleate buffer (10mM pH 7.2). The crude homogenate was centrifuged for 25 min at 10000 g using « Beckmann-Coulter microfuge 20 R centrifuge » and 4°C after incubation. The supernatant was removed, stored at -20°C for future use as crude extract for protein and enzyme assays.

Proteins were quantified according to method described by Bradford (1976) as follows:

- 10 µl of extract were added to 490 µl of distilled water;
- 500 µl of Bradford reagent was added to the mixture;
- The mixture was incubated at 25°C in darkness for 15 min.

The optical density (OD) of protein was read at 595nm using a spectrophotometer (Shimadzu UV-1605 UV-visible) against a blank containing distilled water. For each extract the experiment was carried out in triplets and the protein content was expressed in mg-equivalent of BSA per Fresh Weight (Bradford, 1976). This in reference to the extrapolation of the standard curve obtained under the same conditions as the samples while using BSA (Bovine Serum Albumine) at 0.1 mg/ml.

II.3.5.2. Peroxidase assay

Peroxidase assay in the protein extract was done according to the modified method of Baaziz (1994) with the following steps:

- 925 µl of Tris- Maleate buffer (10mM pH 7.2, containing 1g gaïacol) was put into a reation container;
- 25 µl of protein extract was added to the mixture;
- 50 µl of H_2O_2 (10%) was then added.
- The mixture was incubated for 3 min at 25°C.

Peroxidase activity was determined following the formation of tetragaïacol read at 470 nm using a spectrophotometer (Shimadzu UV-1605 UV-visible) against a blank containing 25 µl of distlled water instead of the protein extract. Peroxidase activity was expressed in enzyme units per Fresh Weight (Abs at 470 nm/min/g of FW) and calculated with the following formula:

$$\text{Enzyme activity} = \frac{\Delta Abs}{\Delta t * FW}$$

where: Abs=Absorbance; t= time; FW= fresh weght; Δ= variation.

II.3.5.3. β-1,3 Glucanase Assay

The activity of β-1,3-glucanases was evaluated in the protein extract according to the modified method described by Leelasuphakul (2006) using 0,0025% laminarin (g/ml), which is a polymer of β-1,3-glucan. Into test tubes the following were successively introduced:

- 90 µl of sodium acetate buffer (0.1M, pH 4 containing 25mg/l of Laminarin);
- 10 µl of protein extract.

After adding the protein extract the mixture was incubated for 10 min at 40°C. 200 µl of 2M HCl is used to stop the reaction. The volume was completed with 900 µl distilled water. The optical density (OD) was read at 540nm with a spectrophotometer (Shimadzu UV-1605 UV-visible) against a blank contaning distilled water instead of the protein extract. The quantity of sugar released was calculated using the standard glucose curve (OD = f (concentration en glucose)) and β-1,3-glucanase activity was expressed in µmole of glucose released/min/g of Fresh Weight.

II.3.5.4. Polyphenol oxidase Assay

Polyphenol oxidase assay was determined in the protein extract according to the method of Vankammenn and Broumer (1994) using cathechin as a substrate. The following reagents were put in tubes successively:

- 500 µl of phosphate buffer (0.66M, pH 7);
- 150 µl of catechin;

- 35 µl of protein extract.

The mixture was incubated at 25°C for 30 s. The change in absorbance was read after 5 min at 330 nm using a spectrophotometer (Shimadzu UV-1605 UV-visible) against a blank where the protein extract was replaced with distilled water. Polyphenol oxidase activity was expressed in D_{330nm} /min/UE/g of Fresh Weight (FW) and enzyme activity was calculated using the following formula:

$$\text{Enzyme activity} = \frac{\Delta Abs}{\Delta t * FW}$$

where: Abs=Absorbance; t= time; FW= fresh weght; Δ= variation.

II.3.5.5. Phenol extraction and assay of total phenol content

Phenolic compounds were extracted according to the modified method of Boudjeko *et al.* (2007). 1 g of cocoyam leaves was ground in chilled mortars with 5 ml of 80% (v/v) methanol at 4°C. After incubation, tubes were centrifuged thrice at 7000 g for 30 min using the «Beckmann-Coulter microfuge 20 R centrifuge». Supernatants were collected each time. The mixture of the three supernatants constituted the crude extract. Total phenols were quantified using the method described by Marigo *et al.* (1973) using the Folin-Ciocalteu reagent. The following reagents were added successively in test tubes:

- 10 µl of alcoholic extract;
- 500 µl of distilled water;
- 75 µl Folin-Ciocalteu reagent;
- 500 µl of 20% sodium carbonate.

The mixture was incubated at 40°C for 20 min and the intensity of the blue colour was determined at 760 nm with a spectrophotometer (Shimadzu UV-1605 UV-visible) against a blank in which the phenolic extract was replaced with distilled water. For each extract 3 repetitions were done. The content of soluble phenolic compounds was expressed in mg-equivalent of gallic acid per Fresh Weight (FW) using the reference of the standard curve obtained with gallic acid (0.1mg/ml).

II.3.5.6. Quantitative flavonoid assay

Flavonoid content was determined according to the modified method of Kramling and Singleton (1969) using formaldehyde and Folin-Ciocalteu reagent. Into the test tubes were added the following reagents successively:

- 400 µl of phenol extract;
- 200 µl of HCl (50%);
- 200 µl of Formaldehyde (8mg/l).

The mixture was incubated at 4°C for 15min and centrifuged at 3000 g for 5 min using a spectrophotometer (Shimadzu UV-1605 UV-visible) and the supernatant collected. The supernatant was then used to assay non flavonoid compounds according to the method of Marigo *et al.* (1973) described above. Flavonoid content was then determined using the following formula:

$T_{flavonoids} = T_{\text{total phenol}} - T_{\text{non flavonoids}}$ $(mg.g^{-1}$ FW).

II.3.6. Qualitative determination of secondary metabolite compound contents from *X. sagittifolium* leaves using HPLC–DAD–(HR) ESI-MS

II.3.6.1. Sample preparation

Aqueous preparation extracts were separately dissolved in HPLC grade methanol in a concentration of 5 mg/ml then filtrated through a syringe-filter-membrane. Aliquots of 5 µl were injected into the LC–DAD/MS Dionex Ultimate 3000 HPLC (Germany), used for performing the analyses.

II.3.6.2. HPLC-MS conditions

High resolution mass spectra were obtained with an OTOF Spectrometer (Bruker, Germany) equipped with a HRESI source and a UV–vis absorbance detector. The spectrometer was operated in positive mode (mass range: 100-1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation using Na Formate as calibrant. Mass spectra were simultaneously acquired using electrospray ionization in the positive ionization mode. The following parameters were used

for experiments: spray voltage of 4.5 kV, capillary temperature of 200 °C. Nitrogen was used as sheath gas (10 l/min). The spectrometer was attached to an Ultimate 3000 (Thermo Fisher, USA) HPLC system consisting of LC-pump, UV traces were measured at 215, 218, 254, 280 and 330 nm and UV spectra-Diode Array Detector- (DAD) were recorded between 190 and 600 nm, auto sampler (injection volume 5 µl) and column oven (35.0 °C). The separations were performed using a Synergi MAX-RP 100A (50x2 mm, 2.5µ particle size) with a H_2O (+0.1 % HCOOH) (A)/acetonitrile (+0.1 % HCOOH) (B) gradient (flow rate 500 µL/min). Samples were analyzed using a gradient program as follows: 95 % A isocratic for 1.5 min, linear gradient to 100 % B over 6 min, after 100 % B isocratic for 2 min, the system returned to its initial condition (90 % A) within 1 min, and was equilibrated for 1 min.

II.3.6.3. Identification of peaks

Identification of all constituents was performed by HPLC–DAD–MS/MS analysis and by comparing the UV, MS spectra and MS/MS fragmentation of the peaks in the samples with those of data reported in the literature of Scifinder data base.

II.4. Statistical analysis

All results obtained were descriptively analysed. The results were presented as graphs, histograms or tables using the Excel Microsoft software 2013. Results were analyzed statistically by comparison of means with one-way ANOVA using Tukeys test and determination of least significant difference (LSD) at $P < 0.05$ with the Software Package SPSS 17.0 in windows. Correlations between different parameters were analysed using sigmaplot version 11 while principal component analysis (PCA) was done by the XLSTAT 2007 Ink version 10.0 software.

CHAPTER III: RESULTS

III. RESULTS

III.1 Physicochemical properties of the soil before planting and after harvest

Physical analysis of the sand, clay and silt content of the soil on the experimental farm before planting revealed 3.47% moisture, 2.93% sand 35.6% silt and 35% clay. This indicates a clay loam textural class. After harvest the physical analysis of soil from the different treatment plots did not show any significant difference in these values and the soil texture remained clay loam after harvest (Table 8).

Table 8: Physical analyses of soil before planting and after harvest

Physical properties (%)	**Farm before planting**	**Treatment plots after harvest**				
		Control	Poultry manure ($20t.ha^{-1}$)	Poultry manure ($30t.ha^{-1}$)	NPK ($120kg.ha^{-1}$)	NPK ($150kg.ha^{-1}$)
Moisture content	3.47^{d}	3.07^{d}	4.38^{b}	5.39^{a}	3.72^{c}	3.77^{c}
Sand	29.3^{a}	28.3^{a}	29.8^{a}	29.1^{a}	29.2^{a}	28.8^{a}
Silt	35.6^{a}	35.5^{a}	34.8^{a}	35.3^{a}	35.6^{a}	35.4^{a}
Clay	35.0^{a}	36.1^{a}	35.3^{a}	35.5^{a}	35.1^{a}	35.7^{a}
Textural class	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam

Chemically, before planting the soil was slightly acidic (pH 5.6) and both poultry manure treatments ($20t.ha^{-1}$ and $30t.ha^{-1}$) after harvest significantly reduced soil acidity (pH 6.6 and pH 6.9). Poultry manure treatments ($20t.ha^{-1}$ and $30t.ha^{-1}$) significantly increased organic and carbon content as compared to all the other treatments. Poultry treatments ($30t.ha^{-1}$) and NPK fertilizer treatments ($150\ kg.ha^{-1}$) increased the soil total nitrogen content as compared to the control treatments after harvest. NPK fertilizer treatments ($150\ kg.ha^{-1}$) significantly increased soil available phosphorus as compared to the control treatments after harvest. Exchangeable cations (Ca, Mg, K and Na) values were more significant in Poultry manure treatments ($20t.ha^{-1}$ and $30t.ha^{-1}$) as compared to the control treatments after harvest. Poultry manure treatments ($20t.ha^{-1}$ and $30t.ha^{-1}$) also recorded the highest cation exchange capacity values after harvest as compared to the other treatments (Table 9).

Table 9: Chemical analyses of soil before planting and after harvest.

Chemical properties	**Farm before planting**	**Treatment plots after harvest**				
		Control	Poultry manure ($20t.ha^{-1}$)	Poultry manure ($30t.ha^{-1}$)	NPK ($120kg.ha^{-1}$)	NPK ($150kg.ha^{-1}$)
pH in Water	5.6^{b}	5.5^{b}	6.6^{a}	6.9^{a}	5.6^{b}	5.4^{b}
pH in KCl	4.4^{b}	4.2^{b}	6.4^{a}	6.8^{a}	4.7^{b}	4.4^{b}
Organic matter (g/kg)	23.44^{c}	21.75^{c}	48.31^{b}	55.85^{a}	23.41^{c}	21.49^{c}
Total N (g/kg)	1.1^{c}	1.1^{c}	1.4^{b}	1.7^{a}	1.5^{b}	1.6^{a}
Total C (g/kg)	13.44^{c}	12.61^{c}	28.02^{b}	32.40^{a}	13.73^{c}	12.26^{c}
Available P (mg/kg)	4.57^{e}	3.91^{e}	57.84^{d}	94.04^{c}	113.5^{b}	129.9^{a}
Exchangeable Ca (cmol/kg)	1.6^{c}	1.62^{c}	1.96^{b}	2.39^{a}	1.91^{b}	1.99^{b}
Exchangeable Mg (cmol/kg)	0.75^{b}	0.69^{c}	0.81^{b}	1.03^{a}	0.75^{b}	0.81^{b}
Exchangeable K (cmol/kg)	0.08^{c}	0.09^{c}	0.14^{b}	0.29^{a}	0.19^{a}	0.23^{a}
Exchangeable Na (cmol/kg)	0.10^{d}	0.10^{d}	0.49^{a}	0.53^{a}	0.21^{c}	0.31^{b}
Cation exchange capacity (cmol/kg)	5.0^{b}	5.0^{b}	6.0^{a}	6.0^{a}	5.6^{b}	5.6^{b}

Key: KCl= Potassium chloride, N= Nitrogen, C= Carbon, P= Phosphorus, Ca= Calcium, Mg= Magnesium, K= Potassium , Na= Sodium.

III.2. Effects of poultry manure and NPK fertilizers on the growth parameters of white and red cv *Xanthosoma sagittifolium* plants.

All growth parameters (plant height, number of leaves per plant and leaf area) analysed were generally more significant in the white cultivar cocoyam (white cv) plants than in the red cultivar cocoyam (red cv) plants for all treatments. After six months of growth there was a significant decrease in all growth parameters for treatments in both white and red cv *X. sagittifolium* plants. The poultry manure treatments ($30t.ha^{-1}$) showed the most significant increase in all growth parameters analysed from two months after planting to six months after planting among all treatments meanwhile within the same growth period the control treatments (0t of Poultry manure ha^{-1} and 0kg of NPK ha^{-1}) recorded the least increase in average plant height and leaf area for both white and red cultivars of the cocoyam plants. At six months after planting white cv cocoyam plants treated with poultry manure ($30t.ha^{-1}$) showed the most significant growth in terms of average height (86cm) (Table 10 and Fig.14) followed by an average height of 77cm for red cv cocoyam plants treated with poultry manure ($30t.ha^{-1}$) (Table 9 and Fig.14). The red cv cocoyam plants showed no significant difference in the average number of leaves for all the different treatments throughout the growth period (Table 11) meanwhile a significant average number of leaves was recorded after six months of growth for white cv cocoyam plants treated with poultry manure ($30t.ha^{-1}$) (Table 10). White cv plants treated with poultry manure ($30t.ha^{-1}$) after six months of growth recorded the most significant average leaf area ($0.88m^2$) (Table 10) while red cv cocoyam plants treated with poultry manure ($30t.ha^{-1}$) recorded an average leaf area of $0.069m^2$ after six months of growth (Table 11).

III.2.1 Growth parameters of white cv *X. saggittifolium* plants

Average height (cm) steadily increased from 2 months after planting to optimum values after 6 months of growth for all treatments in white cv *X. sagittifolium* plants. At 6 months of growth, poultry manure treatments ($30t.ha^{-1}$) recorded the most significant average height value of 86cm height while the control treatments (0t of Poultry manure. ha^{-1} and 0kg of $NPK.ha^{-1}$) recorded the least average height value of 55cm (Fig.14). After 6 months of growth there was a significant decrease in average height for all treatments (Table 10).

All treatments showed a steady increase in the average number of leaves during the first 6 months of growth, with the most significant value of 6 recorded in poultry manure treatments (30t.ha^{-1}) and the least value of 2 recorded in control treatments (0t of Poultry manure. ha^{-1} and 0kg of NPK.ha^{-1}). At 6 months of growth there was a significant decrease in the average number of leaves for all treatments (Table 10). From 2 months after planting to 6 months of growth, there was a significant increase in average leaf area (m^2) in treatments of poultry manure (20t.ha^{-1} and 30t.ha^{-1}) and treatments of NPK fertilizers (120kg.ha^{-1} and 150kg.ha^{-1}). Six months after growth treatments of poultry manure (30t.ha^{-1}) showed the most significant average leaf area value of 0.088m^2 while the least average leaf area of 0.021m^2 was shown by control treatments (0t of Poultry manure. ha^{-1} and 0kg of NPK.ha^{-1}) after 4 months of growth (Table 10). After 6 months of growth there was a significant decrease in the average leaf area for all treatments (Table 10).

A significant decrease in average root number was observed throughout the period of growth in the control treatments (0t of Poultry manure. ha^{-1} and 0kg of NPK.ha^{-1}) and poultry manure (20t.ha^{-1}) treatments. Treatments of NPK fertilizers (120kg.ha^{-1} and 150kg.ha^{-1}) showed a very significant decrease in average root number from average root numbers of 20 and 23 respectively after 2 months of growth to an average root number of 7 after 4 months of growth (Table 10).

III.2.2. Growth parameters of red cv *X. sagittifolium* plants

In red cv *X. sagittifolium* plants average height (cm) steadily increased from 2 months after planting to optimum values after 6 months of growth for all treatments. At 6 months of growth, poultry manure treatments (30t.ha^{-1}) recorded the most significant average height value of 77cm while the control treatments (0t of Poultry manure. ha^{-1} and 0kg of NPK. ha^{-1}) recorded the least average height value of 50cm (Fig. 14). After 6 months of growth there was a significant decrease in average height for all treatments (Table 11).

All treatments showed an increase in the average number of leaves during the first 6 months of growth. At 6 months of growth the most significant value of 5 was recorded in both poultry manure treatments (20t.ha^{-1} and 30t.ha^{-1}) while the value of 4 was recorded in control treatments (0t of Poultry manure ha^{-1} and 0kg of NPK ha^{-1}) and NPK fertilizer treatments (120kg.ha^{-1}). After 6 months of growth there was a significant decrease in the average number of leaves for all treatments (Table 11).

A significant increase in average leaf area (m^2) was recorded in treatments from 2 months after planting to 6months of growth. At 6 months of growth, treatments of poultry manure ($30t.ha^{-1}$) showed the most significant average leaf area value of $0.069m^2$ while control treatments (0t of Poultry manure.ha^{-1} and 0kg of NPK.ha^{-1}) and NPK fertilizer treatments (120kg ha^{-1}) showed an average leaf area of 0.041 m^2. After 6 months of growth there was a significant decrease in the average leaf area for all treatments (Table 11).

There was no significant difference in the average root number after 2 months of growth for all treatments. After 4 months of growth poultry manure treatments ($20t.ha^{-1}$) recorded a very significant decrease in average root number, while the control (0t of Poultry manure ha^{-1} and 0kg of NPK.ha^{-1}), poultry manure ($20t.ha^{-1}$) and NPK fertilizer ($120kg.ha^{-1}$ and $150kg.ha^{-1}$) treatments showed a significant decrease in mean root number after 6 months of growth (Table 11).

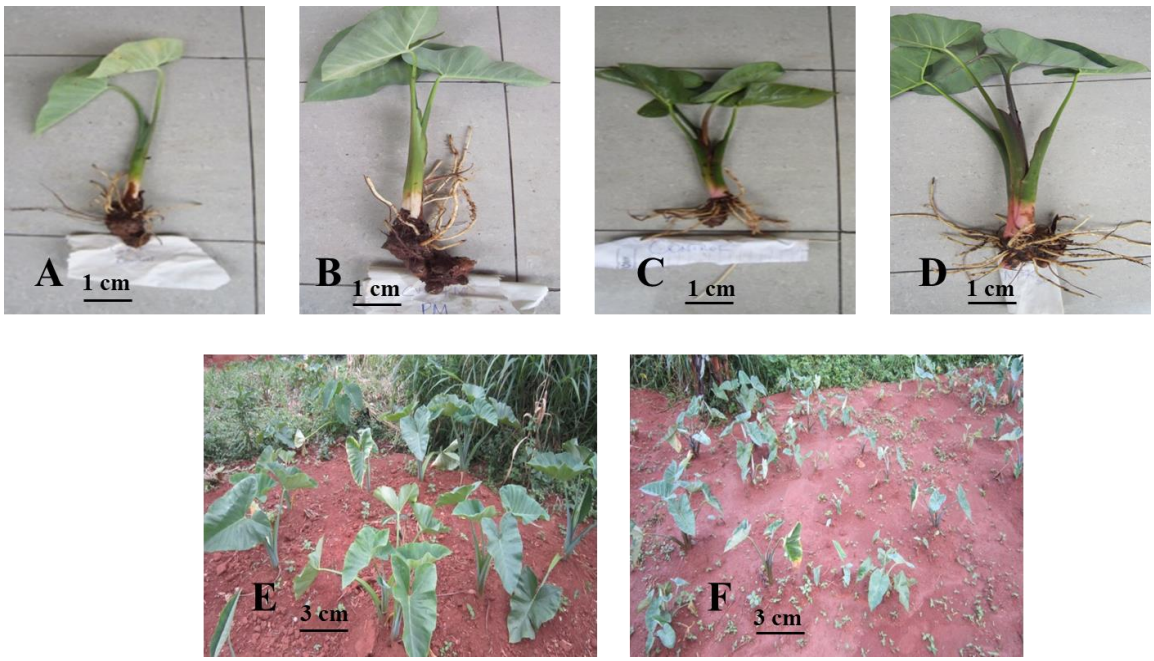


Fig.14. Aspect of cocoyam plant treatments after 6 months of growth: white cv control (A); white cv poultry manure ($30t.ha^{-1}$) (B); red cv control (C); red cv poultry manure ($30t.ha^{-1}$) (D); white cv plot poultry manure ($30t.ha^{-1}$) (E); red cv plot poultry manure ($30t.ha^{-1}$)(F).

Table 10: Growth parameters of white cv *X. sagittifolium* plants

Treatment	**Average plant height** (cm)				**Average number of leaves**				**Average leaf area** (m^2)				**Average root number**			
	2map	**4map**	**6map**	**8map**	**2map**	**4map**	**6map**	**8map**	**2map**	**4map**	**6map**	**8map**	**2map**	**4map**	**6map**	**8map**
Control	35±0.08^{e}	42±0.01^{e}	55±0.09^{e}	25±0.11^{e}	2±0.17^{a}	3±0.12^{ab}	2±0.14^{c}	1±0.16^{a}	0.031±0.01^{d}	0.021±0.01^{e}	0.025±0.01^{e}	0.002±0.01^{e}	21±0.11^{bc}	20±0.08^{b}	15±0.08^{b}	5±0.08^{b}
PM1	70±0.04^{b}	73.7±0.08^{b}	77±0.18^{b}	55±0.02^{b}	3±0.01^{a}	4±0.08^{ab}	5±0.07^{ab}	2±0.10^{a}	0.042±0.01^{b}	0.053±0.03^{b}	0.072±0.01^{b}	0.021±0.01^{d}	22±0.11^{abc}	21±0.08^{ab}	10±0.05^{c}	7±0.06^{ab}
PM2	80±0.11^{a}	86±0.16^{a}	86±0.16^{a}	62±0.07^{a}	4±0.13^{a}	5±0.13^{a}	6±0.17^{a}	3±0.18^{a}	0.051±0.01^{a}	0.067±0.04^{a}	0.088±0.01^{a}	0.031±0.01^{a}	24±0.08^{a}	23±0.18^{a}	26±0.08^{a}	8±0.07^{a}
NPK1	40±0.05^{d}	47.7±0.08^{d}	60±0.11^{d}	39±0.13^{d}	2±0.17^{a}	2±0.11^{b}	3±0.16^{bc}	1±0.07^{a}	0.033±0.01^{d}	0.042±0.01^{d}	0.056±0.01^{d}	0.025±0.01^{c}	20±0.19^{c}	7±0.09^{c}	15±0.08^{b}	6±0.08^{ab}
NPK2	50±0.15^{c}	60±0.13^{c}	70±0.13^{c}	45±0.16^{c}	4±0.11^{a}	4±0.13^{a}	3±0.27^{bc}	1±0.08^{a}	0.039±0.01^{c}	0.048±0.01^{c}	0.061±0.01^{c}	0.028±0.01^{b}	23±0.08^{ab}	7±0.08^{c}	24±0.08^{a}	7±0.08^{ab}
Significance	*	*	*	*	NS	*	*	NS	*	*	*	*	*	*	*	*

KEY: map = months after planting, PM1= Poultry manure (20t.ha^{-1}), PM2 =Poultry manure (30t.ha^{-1}), NPK1 =NPK fertilizer (120Kg.ha^{-1}), NPK2 =NPK fertilizer (150kg.ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability.

Table 11: Growth parameters of red cv *X. sagittifolium* plants

Treatment	**Average plant height** (cm)				**Average number of leaves**				**Average leaf area** (m^2)				**Average root number**			
	2map	**4map**	**6map**	**8map**	**2map**	**4map**	**6map**	**8map**	**2map**	**4map**	**6map**	**8map**	**2map**	**4map**	**6map**	**8map**
Control	23±0.11^{d}	41±0.10^{e}	50±0.01^{e}	40±0.12^{e}	2±0.11^{a}	3±0.01^{a}	4±0.12^{a}	1.5±0.14^{a}	0.020±0.02^{c}	0.031±0.11^{c}	0.041±0.03^{c}	0.018±01^{c}	16±0.1^{a}	15±0.12^{c}	17±0.1^{a}	6±0.13^{b}
PM1	29±0.14^{b}	51±0.18^{b}	60±0.17^{b}	38±0.16^{c}	3±0.11^{a}	3±0.09^{a}	5±0.17^{a}	2±0.18^{a}	0.027±0.02^{b}	0.043±0.01^{b}	0.065±0.03^{b}	0.021±0.01^{b}	17±0.10^{a}	25±0.11^{a}	10±0.13^{c}	8±0.11^{ab}
PM2	35±0.10^{a}	62±0.15^{a}	77±0.01^{a}	59±0.06^{a}	4±0.17^{a}	4.3±0.14^{a}	5±0.16^{a}	3±0.08^{a}	0.034±0.02^{a}	0.054±0.02^{a}	0.069±0.01^{a}	0.031±0.01^{a}	16±0.01^{a}	19±0.11^{b}	16±0.09^{ab}	9±0.01^{a}
NPK1	26±0.08^{c}	46±0.08^{d}	55±0.16^{d}	38±0.09^{c}	2±0.01^{a}	2.7±0.07^{b}	4±0.08^{a}	1.3±0.09^{a}	0.022±0.02^{c}	0.028±0.02^{d}	0.041±0.02^{d}	0.011±0.01^{d}	16±0.05^{a}	13±0.16^{c}	16±0.08^{ab}	7±0.13^{ab}
NPK2	30±0.08^{b}	48±0.08^{c}	58±0.11^{c}	51±0.17^{b}	3±0.11^{a}	3.3±0.12^{a}	4.5±0.1^{a}	1.6±0.19^{a}	0.025±0.01^{b}	0.041±0.01^{c}	0.058±0.02^{c}	0.013±0.01^{d}	17±0.09^{a}	18±0.13^{b}	14±0.12^{b}	6±0.06^{b}
Significance	*	*	*	*	NS	NS	NS	NS	*	*	*	*	*	*	*	*

KEY: map = months after planting, PM1= Poultry manure (20t.ha^{-1}), PM2= Poultry manure (30t.ha^{-1}), NPK1= NPK fertilizer (120Kg.ha^{-1}), NPK2 =NPK fertilizer (150kg.ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability.

III.3. Effects of poultry manure and NPK fertilizers on the yield parameters of white and red cv *Xanthosoma sagittifolium* plants

After a period of 9 months the yield parameters were assessed. The four yield parameters (tuber number per plant, tuber weight, tuber length and tuber girth) analysed after harvest were generally greater in white cv cocoyam plants than in red cv cocoyam plants for all treatments. Poultry manure treatments (30t.ha^{-1}) showed the most significant yield parameters while the control treatments (0t of Poultry manure.ha^{-1} and 0kg of NPK.ha^{-1}) had the least yield parameters for both cultivars. White cv plants treated with poultry manure (30t.ha^{-1}) recorded an average tuber number per plant of 8, an average tuber weight of 250g, an average tuber length of 14cm, and an average girth of 18.5cm (Table 12 and Fig.15) meanwhile red cv plants treated with poultry manure (30t.ha^{-1}) recorded 5.7 as average tuber number per plant, 124.7g as average tuber weight, 8cm as average tuber length and 14.5cm as average tuber girth (Table 13 and Fig. 15).

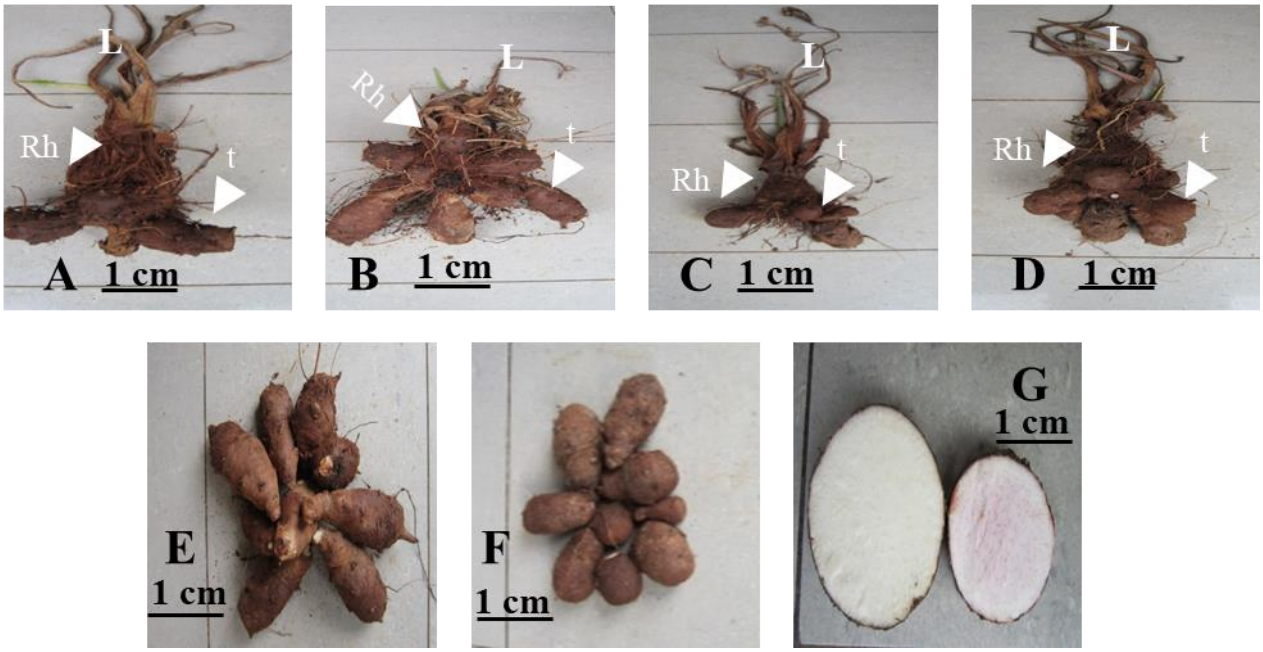


Fig.15. Aspect of cocoyam plants under different fertilizer treatments at harvest (after 9 months): white cv control (A); white cv poultry manure (30t.ha^{-1}) (B); red cv control (C); red cv poultry manure (30t.ha^{-1}) (D); white cv tubers poultry manure (30t.ha^{-1}) (E); red cv tubers poultry manure (30t.ha^{-1}) (F); girth difference of white and red cvs (G). Dead leaves (L), Rhizomes (Rh) and tubers (t).

Table 12: Yield parameters of white cv *X. sagittifolium* plants.

Yield Parameters	TREATMENTS					Significance
	Control	PM1	PM2	NPK1	NPK2	
Tuber numberplant^{-1}	2±0.18^{c}	4±0.18^{bc}	8±0.18^{a}	3±0.18^{c}	6±0.18^{ab}	*
Tuber Weight (g)	20±0.18^{e}	100±0.18^{c}	250±0.18^{a}	80±0.18^{d}	230±0.18^{b}	*
Tuber Length (cm)	6±0.26^{c}	10±0.28^{b}	14±0.29^{a}	7±0.16^{c}	12±0.26^{ab}	*
Tuber Girth (cm)	10.5±0.11^{c}	13.5±0.11^{b}	18.5±0.12^{a}	11.5±0.13^{bc}	17±0.12^{a}	*

KEY: PM1= Poultry manure (20t.ha^{-1}), PM2= Poultry manure (30t.ha^{-1}), NPK1= NPK fertilizer (120Kg.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability using Turkeys test.

Table 13: Yield parameters of red cv *X. sagittifolium* plants

Yield Parameters	TREATMENTS					Significance
	Control	PM1	PM2	NPK1	NPK2	
Tuber number plant^{-1}	2.3±0.20 ^{c}	4±0.38^{b}	5.7±0.37^{a}	2.7±0.40^{bc}	3.6±0.31^{bc}	*
Tuber Weight (g)	22±0.34^{e}	52±0.30^{c}	124.7±0.29^{a}	42±0.27^{d}	94±0.33^{b}	*
Tuber Length (cm)	3±0.21^{b}	5±0.23^{b}	8±0.20^{a}	5±0.20^{b}	8±0.19^{a}	*
Tuber Girth (cm)	6±0.12^{c}	7±0.14^{b}	14.5±0.10^{a}	8±0.16^{b}	13±0.44^{a}	*

KEY: PM1= Poultry manure (20t.ha^{-1}), PM2= Poultry manure (30t.ha^{-1}), NPK1= NPK fertilizer (120Kg.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability using Turkeys test.

III.4. Evaluation of crude protein content and some macromineral nutrient contents in harvested tubers of white and red cv *X. sagittifolium* plants

The crude protein content values in the harvested red tubers were significantly higher than those of the white tubers for the treatments evaluated. In the white cv tubers crude protein content values were more significant in NPK (150kg.ha^{-1}) treatments with 8.34% than in the control (0kg.ha^{-1}) having 4.79%. Red tuber crude protein content value was very significant in in poultry manure (30t.ha^{-1}) treatments with 18.14% and least significant in control (0kg.ha^{-1}) treatments with 7.08% (Table 14). From the results obtained on the macro-mineral nutrient content in harvested tubers, Ca, Mg, Na, P and N contents were more significant in red tubers than the white tubers of *X. sagittifolium* harvested. K was more significant in the white tubers than in the red tubers. There was no significant difference in the Ca and P content for the 3 treatments in white cv tubers. NPK fertilizer (150kg.ha^{-1}) treatments for white cv *X. sagittifolium* tubers recorded more significant values in Mg

(0.042%), K (1.258%) and N (1.337%) than control (0kg.ha^{-1}) tuber treatments with Mg (0.034%), K (0.920%) and N (0.767%). Na content was more significant in poultry manure (30t.ha^{-1}) treated white cv tubers (175.47 ug.g^{-1}) than in control (0kg.ha^{-1}) white cv tubers (116.85 ug.g^{-1})(Table 15).

Results from red cv tubers, showed that NPK fertilizer (150kg.ha^{-1}) treatments had more significant values in Ca (0.050%), Mg (0.081%), K (0.920%) and P (0.090%) than control (0kg.ha^{-1}) tuber treatments with Ca (0.040%) Mg (0.061%), K (0.791%) and P (0.050%). Na content (344.11 ug.g^{-1}) and N content (2.903%) were significantly higher in poultry manure (30t.ha^{-1}) treated red cv tubers than in the control (0kg.ha^{-1}) red cv tubers with Na (197.11 ug.g^{1}) and N (1.134%) (Table 15).

Table 14: Crude protein content in white and red *X. sagittifolium* tubers

Treatment	**White cv tuber Crude protein content %**	**Red cv tuber Crude protein content %**
Control (0kg.ha^{-1})	4.79^{c}	7.08^{c}
Poultry manure (30t.ha^{-1})	6.67^{b}	18.14^{a}
NPK(150kg.ha^{-1})	8.34^{a}	9.09^{b}
Significance at 5% level of probability	*	*

Tukeys test and determination of least significant difference (LSD) at $P < 0.05$

Table 15: Macro-mineral content in the harvested tubers of white and red cv *X. sagittifolium* plants

Treatment	**White cultivar tuber Macro-mineral content**						**Red cultivar tuber Macro-mineral content**					
	Ca %	Mg %	K %	Na ug/g	P %	N %	Ca %	Mg %	K %	Na ug/g	P %	N %
Control(0kg.ha^{-1})	0.020^{a}	0.034^{ab}	0.920^{b}	116.85^{c}	0.074^{a}	0.767^{c}	0.040^{b}	0.061^{b}	0.791^{b}	197.11^{c}	0.050^{c}	1.134^{c}
Poultry manure (30t.ha^{-1})	0.022^{a}	0.038^{a}	1.047^{b}	175.47^{a}	0.066^{a}	1.068^{b}	0.046^{a}	0.063^{b}	0.880^{a}	344.11^{a}	0.069^{b}	2.903^{a}
NPK (150kg.ha^{-1})	0.022^{a}	0.042^{a}	1.258^{a}	164.75^{b}	0.074^{a}	1.337^{a}	0.050^{a}	0.081^{a}	0.920^{a}	330.83^{b}	0.090^{a}	1.607^{b}
Significance at 5% level of probability	NS	*	*	*	NS	*	*	*	*	*	*	*

Tukeys test and determination of least significant difference (LSD) at $P < 0.05$

III.5. Evaluation of some biochemical parameters in white and red cv *X. sagittifolium* leaves under the influence of poultry manure and NPK fertilizers

III.5.1 Effects of poultry manure and NPK fertilizer treatments on protein content in white and red cv *X. sagittifolium* leaves during growth

At 6 months of growth, leaves of poultry manure treated plants (30 $t.ha^{-1}$) expressed the most significant protein content (5.04±0.38mg eq BSA g^{-1} FW) for white cv cocoyam plants followed by the same poultry manure treatments (30 $t.ha^{-1}$) for red cv (4.11±0.52mg eq BSA g^{-1} FW). The Control White cv plants (0t of Poultry manure.ha^{-1} and 0 kg of NPK.ha^{-1}) had the least protein content (2.56±0.29 mg eq BSA g^{-1} FW). The white cv *X. sagittifolium* plants treated with poultry manure (150kg.ha^{-1}) showed a progressive decrease in protein content from 3.6±0.58 mg eq BSA g^{-1} FW at 2 months of growth to 2.46±0.39 mg eq BSA g^{-1} FW at 6 months of growth. There was no significant difference in proten content between the Control treatments (0t of Poultry manure ha^{-1} and 0kg of NPK.ha^{-1}) and NPK fertilzer treatments (150kg.ha^{-1}) in red cv *X. sagittifolium* plants after 6 months of growth (Table 16).

III.5.2. Effects of poultry manure and NPK fertilizer treatments on peroxidase activity in white and red cv *X. sagittifolium* leaves during growth

Peroxidase activity increased in the three treatments (Control, Poultry manure 30t ha^{-1} and NPK 150kg.ha^{-1}) for white cv *X. sagittifolium* plants from 2 months after planting to 6 months after planting with Poultry manure 30t.ha^{-1} recording the most significant value of 4.89±1.36 UE min^{-1} at 6 months. At 6 months of growth white cv *X. sagittifolium* plants treated with NPK fertilizer (150kg.ha^{-1}) recorded the least peroxidase activity value of 1.30±1.92 UE min^{-1} (Table 17).

The Control (0t of Poultry manure.ha^{-1} and 0kg of NPK.ha^{-1}) and NPK fertilizer (150kg.ha^{-1}) treatments for red cv *X. sagittifolium* plants recorded an increase in peroxidase activity between 2 and 4 months after planting, followed by a significant decrease at 6 months. NPK fertilizer (150kg.ha^{-1}) treatments for red cv *X. sagittifolium* plants showed the most significant peroxidase activity after 4 months of growth with a value of 2.34±0.55UE min^{-1} while the control (0t of Poultry manure.ha^{-1} and 0kg of NPK.ha^{-1}) treatments had the least peroxidase activity value of 0.12±0.09UE min^{-1} at 2 months of growth (Table 17).

Table 16: Protein content (mg eq BSA/g FW) in white and red cv *X. sagittifolium* leaves during growth

	White cv			Red cv		
TREATMENT	**2 map**	**4 map**	**6map**	**2 map**	**4map**	**6map**
CONTROL	2.87 ± 0.39^{c}	4.32 ± 0.25^{a}	2.56 ± 0.29^{b}	3.27 ± 0.14^{b}	3.1 ± 0.51^{a}	3.1 ± 0.47^{b}
PM2	4.57 ± 0.25^{a}	4.67 ± 0.29^{a}	5.04 ± 0.38^{a}	3.99 ± 0.51^{a}	3.4 ± 0.48^{a}	4.11 ± 0.52^{a}
NPK2	3.6 ± 0.58^{b} *	3.0 ± 0.14^{b} *	2.46 ± 0.39^{b} *	3.3 ± 0.52^{b} *	3.58 ± 0.47^{a} Ns	3.1 ± 0.16^{b} *

KEY: MAP = Months after planting, PM2= Poultry manure ($30t.ha^{-1}$), NPK2=NPK fertilizer ($150kg.ha^{1}$), NS= Not Significant , * = Significant at 5% level of probability using Turkeys test.

Table 17: Peroxidase activity (UE/min) in white and red cv *X. sagittifolium* leaves during growth

	White cv			Red cv		
TREATMENT	**2 MAP**	**4 MAP**	**6MAP**	**2 MAP**	**4MAP**	**6MAP**
CONTROL	0.41 ± 0.23^{b}	0.90 ± 0.13^{a}	2.35 ± 0.29^{b}	0.12 ± 0.09^{c}	1.62 ± 0.79^{b}	0.85 ± 0.73^{b}
PM2	1.16 ± 0.44^{a}	0.42 ± 0.29^{b}	4.89 ± 1.36^{a}	1.15 ± 0.48^{a}	1.26 ± 0.54^{c}	1.58 ± 0.52^{a}
NPK2	0.84 ± 0.58^{ab} *	0.86 ± 0.68^{a} *	1.30 ± 1.92^{b} *	0.49 ± 0.58^{b} *	2.34 ± 0.55^{a} *	1.58 ± 0.16^{a} *

KEY: MAP = Months after planting, PM2= Poultry manure ($30t.ha^{-1}$), NPK2=NPK fertilizer ($150kg.ha^{-1}$), NS= Not Significant,*= Significant at 5% level of probability using Turkeys t

III.5.3. Effects of poultry manure and NPK fertilizer treatments on glucanase activity in white and red cv *X. sagittifolium* leaves during growth

Control (0t of Poultry manure.ha^{-1} and 0kg of NPK.ha^{-1}) and NPK fertilizer (150kg.ha^{-1}) treatments for white cv *X. sagittifolium* plants recorded an increase in glucanase activity between 2 and 4 months after planting, followed by a significant decrease at 6 months. Similarly Control (0t of Poultry manure.ha^{-1} and 0kg of NPK.ha^{-1}) treatments for red cv *X. sagittifolium* plants showed increase in glucanase activity between 2 and 4 months after planting, followed by a significant decrease at 6 months while NPK fertilizer (150kg.ha^{-1}) treatments for red cv *X. sagittifolium* plants illustrated a significant decrease in glucanase activity from 6.67±0.03 mg eq glucose g^{-1} FW to 1.34±0.24 mg eq glucose g^{-1} FW between 2 and 4 months after planting followed by an increase to 4.94±0.04 mg eq glucose g^{-1} FW at 6months of growth (Table 18).

The most significant value of glucanase activity was observed in red cv cocoyam plants treated with Poultry manure (30t.ha^{-1}), 9.33±1.17 mg eq glucose g^{-1} FW meanwhile white cv cocoyam plants treated with Poultry manure (30t.ha^{-1}), recorded 7.36±0.28mg eq glucose g^{-1} FW after six months of planting. The least value for glucanase activity was seen in red cv plants treated with NPK (150kg.ha^{-1}), 1.34±.24 mg eq glucose g^{-1} FW after 4 months of planting (Table 18).

III.5.4 Effects of poultry manure and NPK fertilizer treatments on polyphenol oxidase activity in white and red cv *X. sagittifolium* leaves during growth

The control (0t of Poultry manure ha^{-1} and 0kg of NPK.ha^{-1}) and NPK fertilizer (150kg.ha^{-1}) treatments for both white and red cv *X. sagittifolium* plants recorded an increase in polyphenol oxidase activity between 2 and 4 months after planting, followed by a significant decrease at 6 months of growth. Poultry manure (30t.ha^{-1}) treatments for both white and red cv *X. sagittifolium* plants showed a decrease in polyphenol oxidase activity between 2 and 4 months after planting, followed by a significant increase at 6 months of growth. (Table 19).

At 6 months of growth, polyphenol oxidase activity was most significant in white cv cocoyam plants treated with NPK (150kg.ha^{-1}), 4.99±0.10 in D330 nm^{-1} min^{-1}UE^{-1}g^{-1} FW while the red cv Control (0t of Poultry manure.ha^{-1} and 0kg of NPK.ha^{-1}), Poultry manure (30t.ha^{-1}) and NPK (150kg.ha^{-1}) treatments showed no significant difference in polyphenol

oxidase. Red cv cocoyam plants treated with Poultry manure (30t.ha^{-1}) recorded a polyphenol oxidase activity of 4.39±0.33 in D330 nm^{-1} min^{-1} UE^{-1} g^{-1} FW after 4 months of planting (Table 19).

Table 18: Glucanase activity (mg eq glucose/g FW) in white and red cv *X. sagittifolium* leaves during growth

	White cv			Red cv		
TREATMENT	**2 MAP**	**4 MAP**	**6MAP**	**2 MAP**	**4MAP**	**6MAP**
CONTROL	2.21±0.11^{b}	4.19±0.55^{a}	1.82±0.29^{c}	5.73±0.03^{b}	6.19±0.08^{a}	4.41±0.71^{b}
PM2	7.26±0.95^{a}	6.63±0.08^{a}	7.36±0.28^{a}	5.51±0.26^{b}	5.23±0.18^{b}	9.33±1.17^{a}
NPK2	1.60±0.07^{b}	4.19±0.55^{b}	3.55±0.23^{b}	6.67±0.03^{a}	1.34±0.24^{c}	4.94±0.04^{b}
	*	*	*	*	*	*

KEY: MAP = Months after planting, PM2= Poultry manure (30t.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{1}), NS= Not Significant, * = Significant at 5% level of probability using Turkeys test.

Table 19: Polyphenol oxidase (ppo) activity (in D330/nm/min/UE/g MF)) in white and red cv *X. sagittifolium* leaves during growth

	White cv			Red cv		
TREATMENT	**2 MAP**	**4 MAP**	**6MAP**	**2 MAP**	**4MAP**	**6MAP**
CONTROL	3.03±0.07^{c}	2.74±0.23^{b}	4.42±0.25^{b}	3.00±0.10^{a}	2.76±0.19^{b}	3.07±0.09^{a}
PM2	3.39±0.06^{b}	4.42±0.25^{a}	3.12±0.13^{c}	2.31±0.05^{c}	4.39±0.33^{a}	2.96±0.08^{a}
NPK2	4.05±0.22^{a}	2.41±0.25^{b}	4.99±0.10^{a}	2.65±0.07^{b}	1.47±0.09^{c}	3.07±0.10^{a}
	*	*	*	*	*	NS

KEY: MAP = Months after planting, PM2= Poultry manure (30t.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability using Turkeys tes

III.5.5. Effects of poultry manure and NPK fertilizer treatments on phenolic content in white and red cv *X. sagittifolium* leaves during growth

The control (0t of Poultry manure ha^{-1} and 0kg of NPK.ha^{-1}) and poultry manure (150t.ha^{-1}) treatments for both white cv *X. sagittifolium* plants illustrated a non significant decrease in phenolic contents between 2 and 6 months after planting while control (0t of Poultry manure ha^{-1} and 0kg of NPK.ha^{-1}) and poultry manure (150t.ha^{-1}) red cv *X. sagittifolium* plants showed a significant increase in phenol content between 2 and 4 months after planting followed by a decrease in phenol content at 6 months of growth. (Table 20).

NPK (150kg.ha^{-1}) treatments recorded the most significant values in phenol contents in both cultivars. White cv *X. sagittifolium* plants had 1.22±0.13 mg eq catechin g^{-1} FW and red cv cocoyam plants had 1.12±0.11mg eq catechin g^{-1} FW after 2 months of planting. The white cv cocoyam plants showed no significant difference in phenolic contents in the Control, Poultry manure (30t.ha^{-1}) and NPK (150kg.ha^{-1}) treatments after 4 months of planting (Table 20).

III.5.6. Effects of poultry manure and NPK fertilizer treatments on flavonoid content in white and red cv *X. sagittifolium* leaves during growth

Control (0t of Poultry manure.ha^{-1} and 0kg of NPK.ha^{-1}) white cv *X. sagittifolium* plants showed a significant increase in flavonoid content between 2 and 6 months while in control red cv *X. sagittifolium* plants, flavonoid content increased between 2 and 4 months followed by a significant decrease at 6 months of growth. Poultry manure (150kg.ha^{-1}) white cv plants recorded a significant decrease in flavonoid content between 2 and 4 months of planting. This was followed by a significant increase at 6 months of growth. Poultry manure (150kg.ha^{-1}) red cv plants depicted a significant increase in flavonoid content between 2 and 4 months of growth followed by a significant decrease at 6 months of growth (Table 21).

Flavonoid content was greatest at 2 months after planting in red cv cocoyam plants treated with NPK (150kg.ha^{-1}), 1.08±0.16mg g^{-1} FW while white cv cocoyam plants treated with Poultry manure (30t.ha^{-1}) also recorded a significant flavonoid content value of 0.96±0.03mg g^{-1} FW after 6 months of growth. The least flavonoid content value was

recorded by the control red cv *X. sagittifolium* plants, 0.06±0.01 mg g^{-1} FW after 6 months of growth (Table 21).

Table 20: Phenolic content (mg eq catechin/g FW) in white and red cv *X. sagittifolium* leaves during growth

	White cv			Red cv		
TREATMENT	**2 MAP**	**4 MAP**	**6MAP**	**2 MAP**	**4MAP**	**6MAP**
CONTROL	0.93 ± 0.04^{b}	0.91 ± 0.11^{a}	0.86 ± 0.13^{b}	0.45 ± 0.02^{c}	0.76 ± 0.14^{ab}	0.66 ± 0.01^{ab}
PM2	0.97 ± 0.03^{b}	0.98 ± 0.03^{a}	0.81 ± 0.04^{b}	0.56 ± 0.01^{b}	1.00 ± 0.04^{a}	0.55 ± 0.14^{b}
NPK2	1.22 ± 0.13^{a}	0.76 ± 0.14^{a}	1.13 ± 0.07^{a}	1.12 ± 0.11^{a}	0.47 ± 0.04^{b}	0.83 ± 0.12^{a}
	*	NS	*	*	*	*

KEY: MAP = Months after planting, PM2= Poultry manure (30t.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability using Turkeys test.

Table 21: Flavonoid content (mg/g FW) in white and red cv *X. sagittifolium* leaves during growth

	White cv			Red cv		
TREATMENT	**2 MAP**	**4 MAP**	**6MAP**	**2 MAP**	**4MAP**	**6MAP**
CONTROL	0.25 ± 0.06^{c}	0.39 ± 0.02^{b}	0.56 ± 0.08^{b}	0.28 ± 0.01^{b}	0.43 ± 0.04^{b}	0.06 ± 0.01^{b}
PM2	0.73 ± 0.03^{a}	0.1 ± 0.02^{c}	0.96 ± 0.03^{a}	0.11 ± 0.03^{b}	0.86 ± 0.03^{a}	0.37 ± 0.01^{a}
NPK2	0.46 ± 0.08^{b}	0.56 ± 0.03^{a}	0.38 ± 0.03^{c}	1.08 ± 0.16^{a}	0.36 ± 0.03^{c}	0.38 ± 0.05^{a}
	*	*	*	*	*	*

KEY: MAP = Months after planting, PM2= Poultry manure (30t.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), NS= Not Significant , * = Significant at 5% level of probability using Turkeys test.

III.6. Effects of poultry manure and NPK fertilizers on flavonoid and other metabolite profiles in white and red cv cocoyam (*Xanthosoma sagittifolium*) L. leaves during growth

III.6.1. Spectra analyses

The results obtained enabled the detection of 16 compounds among which 8 flavone C- glycosides which were tentatively identified on the basis of their mass spectra (MS) and ultra violet (UV) spectra, and comparisons with standards when available, as well as with literature data. The other 8 compounds detected were not characterized because their UV and MS spectra as well as the literature data did not provide sufficient evidence for their tentative identification (Table 22).

Flavonoids were determined in the fluorescent region of the UV spectra, which represented the first four peaks with varying intensities identified in the spectra of all treatments. In the first peak two metabolites with a molecular formula $C_{27}H_{30}O_{15}$ and an average molecular weight of 595.16g/mol were identified. 6,8-Di-C-glucopyranosylapigenin was identified with a retention time of 2.63 min with 3 absorption wavelengths peaks (210 nm,270 nm and 335 nm) (Fig.19), while Isovitexin 6"-O-glucopyranoside had a retention time of 2.72 min with 3 absorption wavelength peaks (214 nm, 270 nm and 335 nm) (Fig.20) (Table.22).

In the second peak three metabolites with a molecular formula of $C_{26}H_{28}O_{14}$ and an average molecular weight of 565.15 $g.mol^{-1}$were recorded. The first metabolite on this peak Apigenin 6-C-glucoside 8-C-arabinoside (Schaftoside) had a retention time of 2.82 min with 3 absorption wavelength peaks (214 nm, 270 nm and 235 nm) (Fig.21). The second metabolite Apigenin-6-C-pentoside-8-C- hexoside (Isomer 1) with a retention time of 2.84 min and 3 absorption wavelength peaks (214 nm, 270 nm and 339 nm) (Fig.22) while the third metabolite Apigenin-6-C-pentoside-8-C- hexoside (Isomer 2) had a retention time of 2.90 min. with 3 absorption wavelength peaks (214 nm, 270 nm and 335 nm) (Fig.23) (Table.22). The third peak indicated two metabolites having a molecular formula of $C_{21}H_{20}O_{10}$ and average molecular weight of 433.11 $g.mol^{-1}$. 8-C-Glucosylapiginin (vitexin) had a retention time of 2.97min with 3 absorption wavelength peaks (210 nm, 270 nm and 339 nm) (Fig.24) while 6-C-Glucosylapiginin (isovitexin) recorded a retention time of 3.06

min with 3 absorption wavelength peaks (210 nm, 270 nm and 335 nm) (Fig.25) (Table 22). One metabolite was identified at the fourth peak with a molecular formula of $C_{24}H_{22}O_{13}$ and average molecular weight 519.11g.mol^{-1}. 2"-O-Malonylvitexin recorded a retention time of 3.13 min with 3 absorption wavelength peaks (210nm, 270nm and 335nm) (Fig.26) (Table 22).

Table 22: Spectra analyses

	Rt (min)	**[M+H]$^+$**			**UV, λ_{max} (nm)**	**Formula**	**Metabolites**
		Exp.	**Calcd.**	**Δ(*ppm*)**			
01	2.63	595.1690	595.1557	2.4	210 270 335	$C_{27}H_{30}O_{15}$	6,8-Di-C-glucopyranosylapigenin
02	2.72				214 270 335		Isovitexin 6"-O- glucopyranoside
03	2.82	565.1585	565.1552	2.1	214 270 335	$C_{26}H_{28}O_{14}$	Apigenin 6-C-glucoside 8-C-arabinoside
04	2.84				214 270 339		**Apigenin-6-C-pentoside-8-C- hexoside (Isomer 1)**
05	2.90				214 270 335		**Apigenin-6-C-pentoside-8-C- hexoside (Isomer 2)**
06	2.97	433.1152	433.1129	3.9	210 270 339	$C_{21}H_{20}O_{10}$	8-C-Glucosylapigenin (vitexin)
07	3.06				210 270 335		6-C-Glucosylapigenin (Isovitexin)
08	3.13	519.1169	519.1133	0.8	210 270 335	$C_{24}H_{22}O_{13}$	2"-O-Malonylvitexin
09	3.60	351.2161	351.2166	1.6	218	$C_{18}H_{32}O_5Na$	Not Characterized
10	3.72	353.2321	353.2298	0.2	218	$C_{18}H_{34}O_5Na$	Not Characterized
11	4.13	333.2055	333.2060	1.5	218	$C_{20}H_{28}O_4$	Not Characterized
12	4.22	437.1938	437.1935	0.7	218	$C_{24}H_{30}O_6Na$	Not Characterized
13	4.42	317.2091	317.2087	1.2	222	$C_{18}H_{30}O_3Na$	Not Characterized
14	4.71	537.3077	537.3058	1.9	218	$C_{29}H_{44}O_9$	Not Characterized
15	4.94	619.2894	619.2902	1.2	218	$C_{36}H_{42}O_9$	Not Characterized
16	5.16	527.2401	527.2404	0.5	194	$C_{31}H_{36}O_6Na$	Not Characterized

III.6.1.1. MS Spectra analyses

A systematic study of the obtained MS spectra and the observed fragmentation showed that 8 out of the 16 detected compounds were C- glycosides of apigenin, while the other 8 detected compounds were not characterized.

The MS spectra of the first identified compound (6,8-Di-C-glucopyranosylapigenin**)** recorded 5 fragment signals (m/z) (548.2392,565.1447,595.1534,617.1350 and 633.1176) with a base peak at 595.1534 (most stable fragment) and a molecular ion peak at 633.1176 (Fig.16).

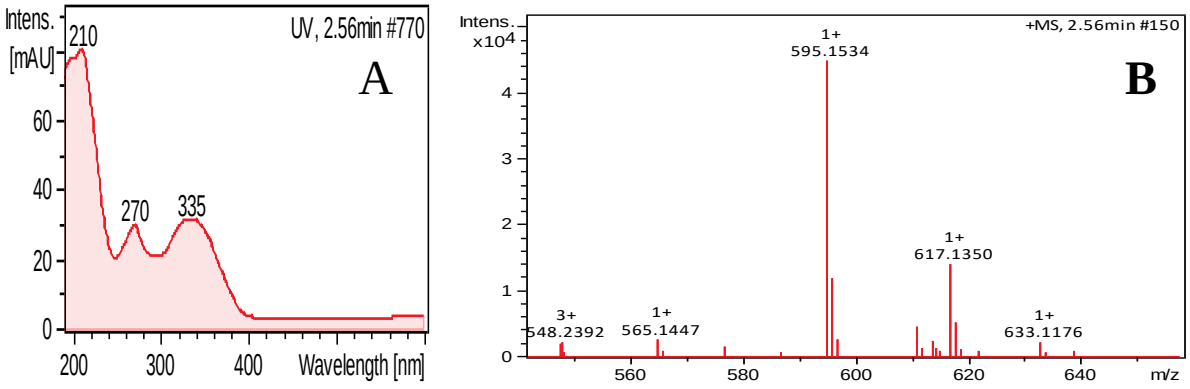


Fig.16. Absorption peaks (A) and MS spectra (B) for $C_{27}H_{30}O_{15}$ (6,8-Di-C-glucopyranosylapigenin)

The MS spectra of the second identified compound (Isovitexin 6"-O-glucopyranoside) recorded 3 fragment signals (m/z) (565.1430,595.1531 and 617.1341) with a base peak at 595.1531 and a molecular ion peak at 633.1176 (Fig.17).

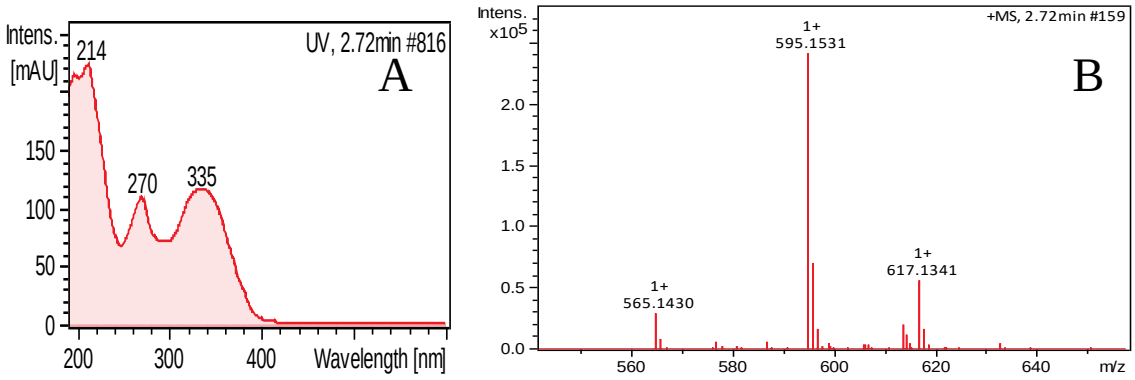


Fig.17. Absorption peaks (A) and MS spectra (B) for $C_{27}H_{30}O_{15}$ (Isovitexin 6"-Oglucopyranoside)

MS spectra of the third identified compound (Apigenin 6-C-glucoside 8-C-arabinoside) showed 3 fragment signals (m/z) (565.1435,587.1240 and 595.1528) with a base peak at 565.1435 and a molecular ion peak at 595.1528 (Fig.18).

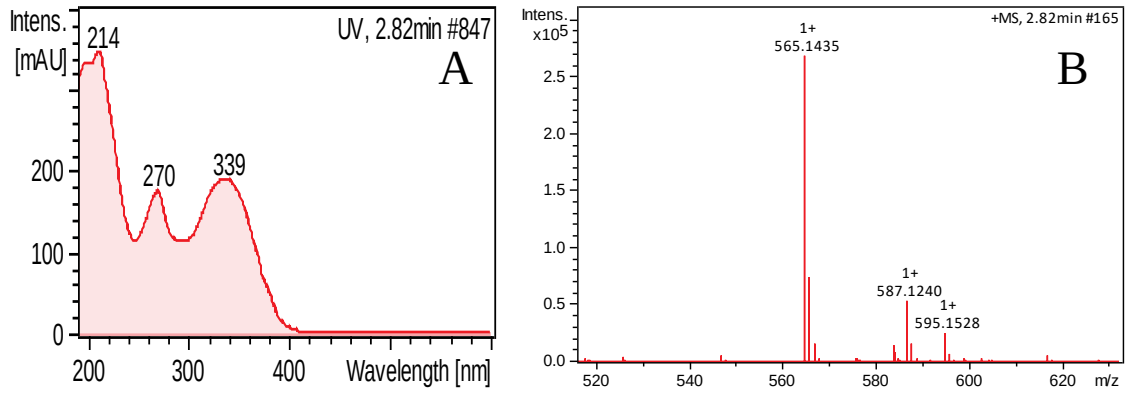


Fig.18. Absorption peaks(A) and MS spectra(B) for $C_{26}H_{28}O_{14}$ (Apigenin 6-C-glucoside 8-arabinoside).

The fourth identified compound (Apigenin-6-C-pentoside-8-C- hexoside (Isomer 1)) showed 2 signal fragments (m/z) (565.1587 and 587.1404) on the MS spectra with a base peak signal value of 565.1587 and a molecular ion signal value of 587.1404 (Fig. 19).

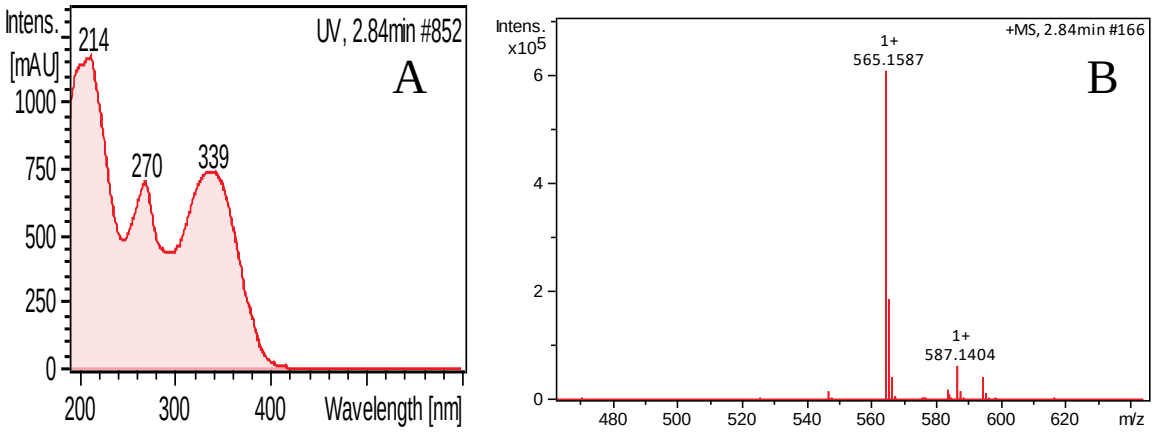


Fig.19. Absorption peaks (A) and MS spectra (B) for $C_{26}H_{28}O_{14}$ (Apigenin-6-C-pentoside-8-C- hexoside (Isomer 1)).

The MS spectra of the fifth identified compound (Apigenin-6-C-pentoside-8-C-hexoside (Isomer 2)) recorded 3 fragment signals (m/z) (518.1104, 565.1588 and 587.1399) with a base peak at 565.1588 and a molecular ion peak at 587.1399 (Fig.20).

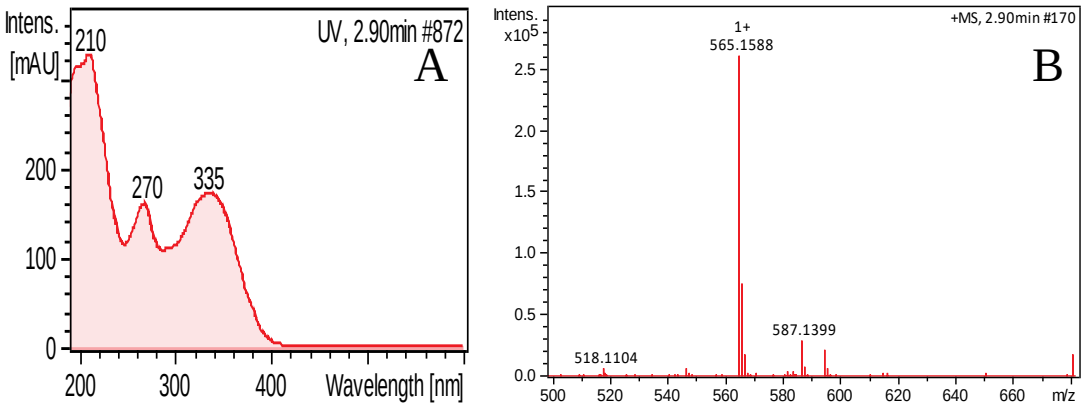


Fig.20. Absorption peaks (A) and MS spectra (B) for $C_{26}H_{28}O_{14}$ (Apigenin-6-C-pentoside-8-C- hexoside (Isomer 2).

The sixth identified compound (8-C- Glycosylapiginin) showed 3 fragment signals (m/z) (415.0972, 433.1080 and 455.0898) on the MS spectra with a base peak of 433.1080 and a molecular ion peak of 455.08998 (Fig. 21).

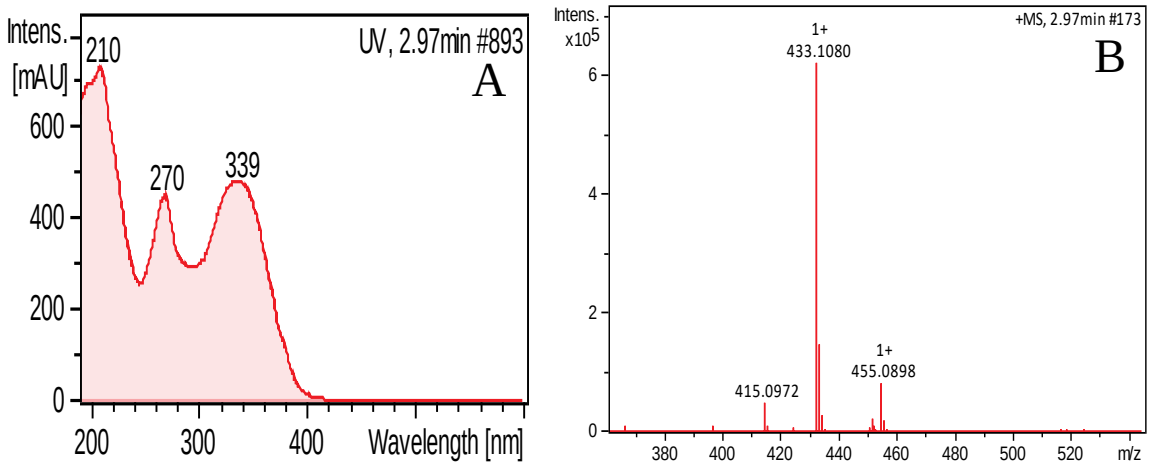


Fig.21. Absorption peaks(A) and MS spectra (B) for $C_{21}H_{20}O_{10}$ (8-C-Glycosylapiginin).

6-C-Glycosylapiginin which was the seventh identified compound, recorded four fragment signals (m/z) (415.1044,433.1152,455.0969 and 519.1164) with a base peak of 433.1152 and a molecular ion peak of 519.1164 (Fig.22).

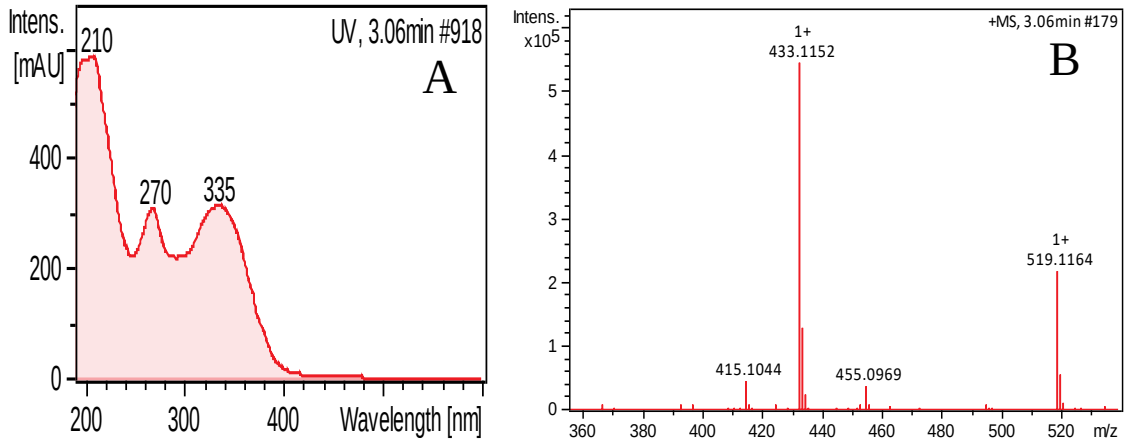


Fig. 22. Absorption peaks (A) and MS spectra (B) for $C_{21}H_{20}O_{10}$ (6--C-Glycosylapigginin).

The eight identified compound (2"-O-Malonylvitexin) showed four fragment signals (m/z) (519.1166, 541.0980, 603.2062 and 650.8446) with a base peak of 519.1166 and a molecular ion peak of 650.8446 (Fig.23).

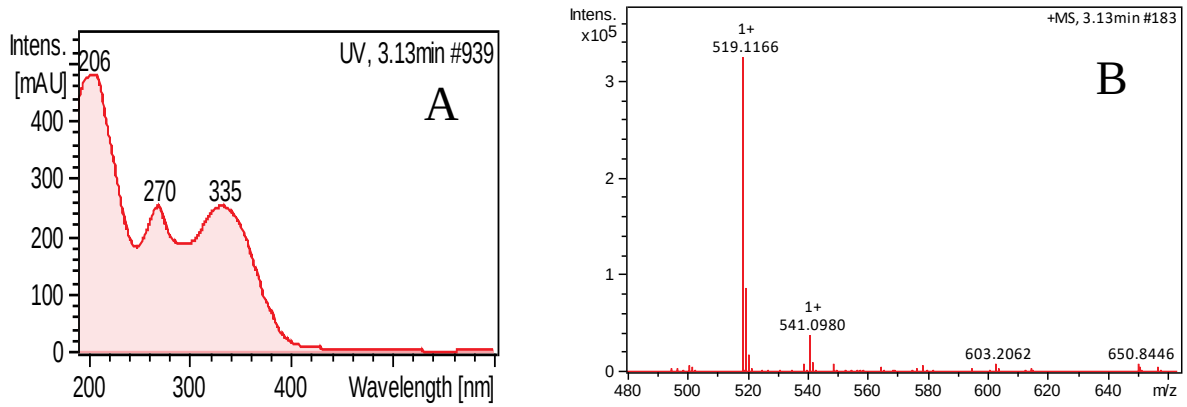


Fig.23. Absorption peaks (A) and MS spectra (B) for $C_{24}H_{22}O_{13}$ (2"-O-Malonylvitexin).

The ninth detected compound had a molecular formula of $C_{18}H_{32}O_5Na$ (Not characterized). This compound recorded a maximum absorption wavelength at 218 nm and 4 signal fragments (m/z) on the MS spectra (301.0721, 311.2222, 351.2158 and 367.1843) with a base peak of 351.2158 and a molecular ion peak of 367.1843 (Fig.24).

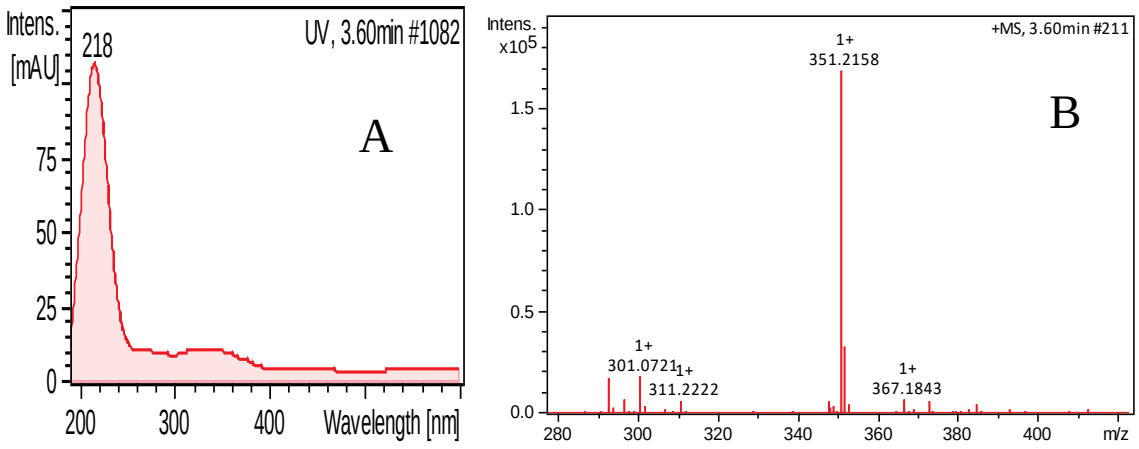


Fig. 24. Absorption peaks (A) and MS spectra (B) for $C_{18}H_{32}O_5Na$ (Not characterized).

The tenth detected compound had a molecular formula of $C_{18}H_{34}O_5Na$ (Not characterized). This compound recorded a maximum absorption wavelength at 218 nm and 3 signal fragments (m/z) on the MS spectra (313.2393, 353.2318, and 375.2138) with a base peak of 353.2318and a molecular ion peak of 375.2138 (Fig.25).

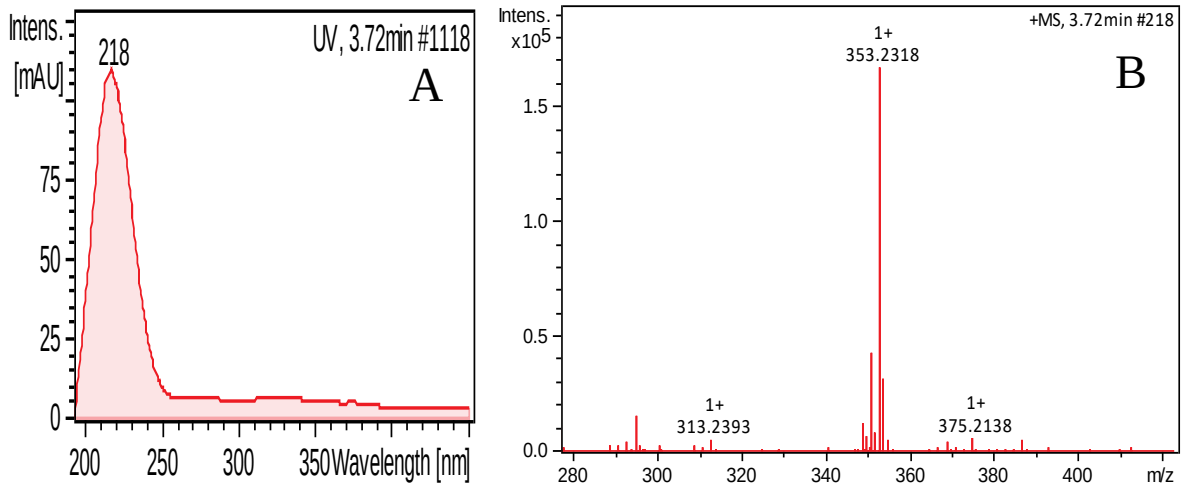


Fig. 25. Absorption peaks (A) and MS spectra (B) for $C_{18}H_{34}O_5Na$ (Not characterized).

The eleventh detected compound had a molecular formula of $C_{20}H_{18}O_4$ (Not characterized). This compound recorded a maximum absorption wavelength at 218 nm and 3 signal fragments (m/z) on the MS spectra (293.2108, 333.2055, and 355.1875) with a base peak of 333.2055 and a molecular ion peak of 355.1875 (Fig.26).

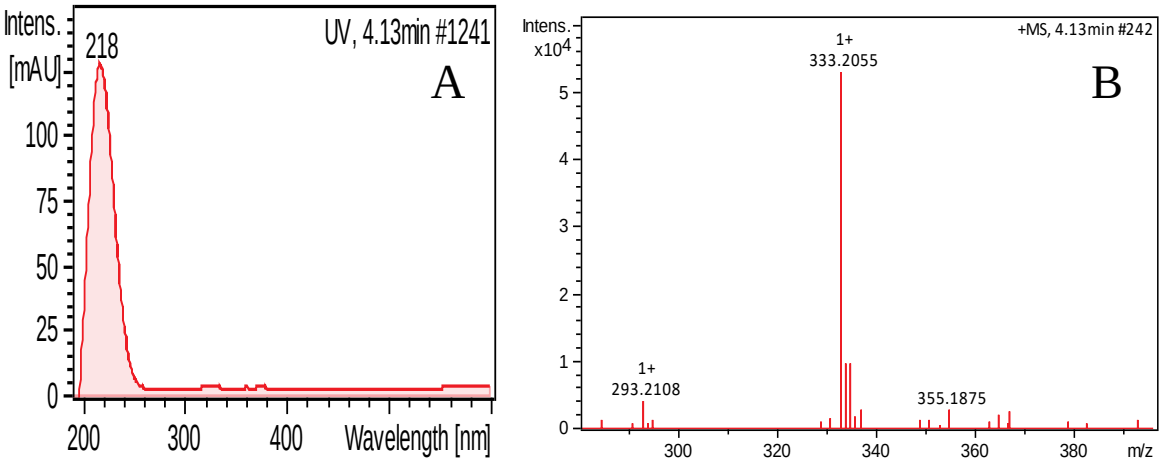


Fig.26. Absorption peaks (A) and MS spectra (B) for $C_{20}H_{28}O_4$ (Not characterized).

Compound twelve which was detected and not characterized had a molecular formula of $C_{24}H_{30}O_6Na$. This compound recorded a maximum absorption wavelength at 218 nm and 2 signal fragments (m/z) on the MS spectra (437.1958 and 453.1697) with a base peak of 437.1958 and a molecular ion peak of 453.1697 (Fig.27).

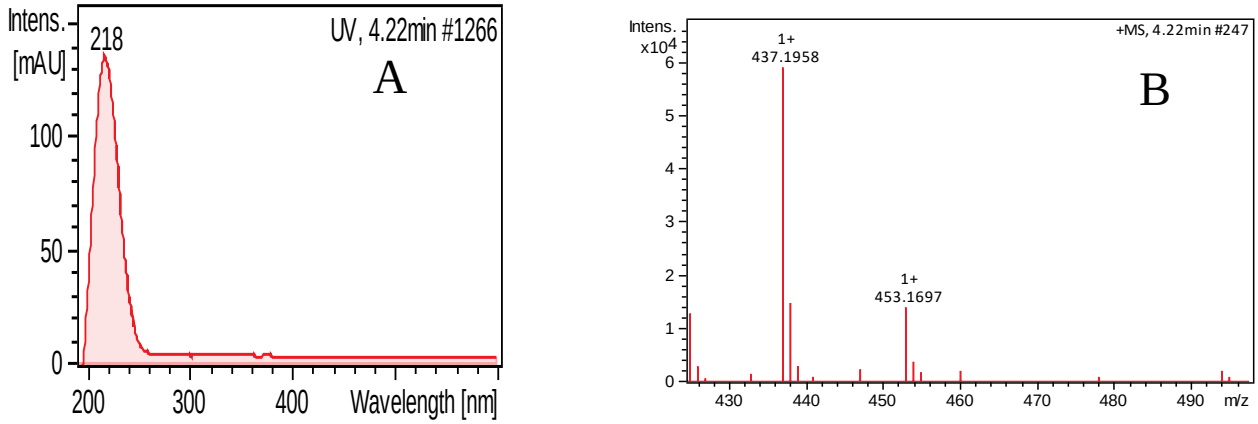


Fig.27. Absorption peaks (A) and MS spectra (B) for $C_{24}H_{30}O_6Na$ (Not characterized).

Compound thirteen which was detected and not characterized had a molecular formula of $C_{18}H_{30}O_3Na$. This compound recorded a maximum absorption wavelength at 222 nm and 4 signal fragments (m/z) on the MS spectra (299.1922, 317.2106,339.1918 and 349.1987) with a base peak of 317.2106 and a molecular ion peak of 349.1987 (Fig.28).

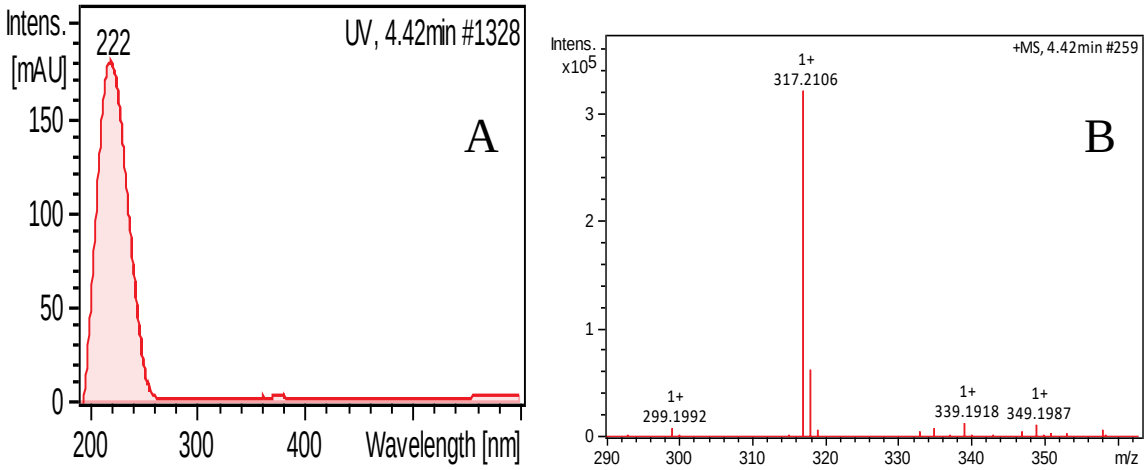


Fig.28. Absorption peaks(A) and MS spectra (B) for $C_{18}H_{30}O_3Na$ (Not characterized).

Compound fourteen which was detected and not characterized had a molecular formula of $C_{29}H_{44}O_9$. This compound recorded a maximum absorption wavelength at 218 nm and 4 signal fragments (m/z) on the MS spectra (425.2171,497.2143, 537.3077 and 701.3742) with a base peak of 537.3077 and a molecular ion peak of 701.3742 (Fig.29).

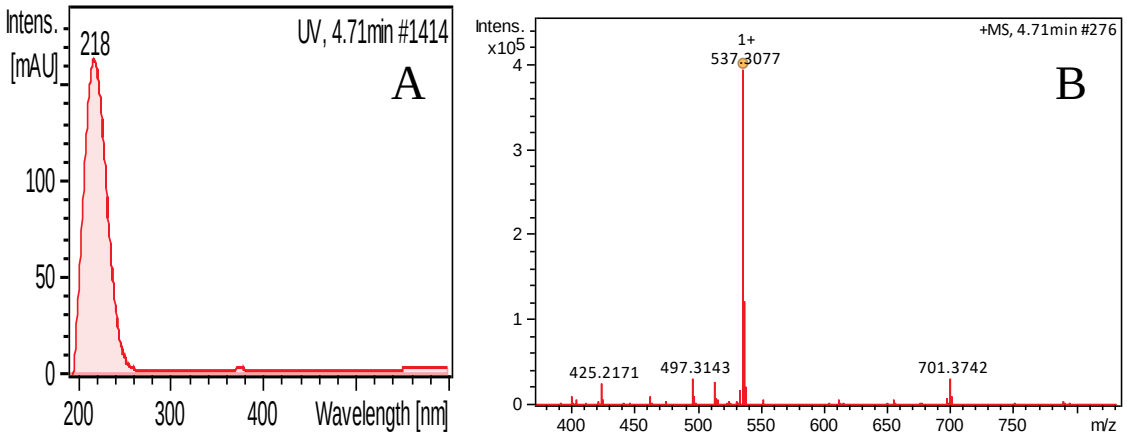


Fig. 29. Absorption peaks (A) and MS spectra (B) for $C_{29}H_{44}O_9$ (Not characterized).

Compound fifteen which was detected and not characterized had a molecular formula of $C_{36}H_{46}O_9$. This compound recorded a maximum absorption wavelength at 218 nm and 5

signal fragments (m/z) on the MS spectra (619.2894, 641.2717, 677.3745, 699.2426 and 701.3742) with a base peak of 537.3077 and a molecular ion peak of 721.2229 (Fig.30).

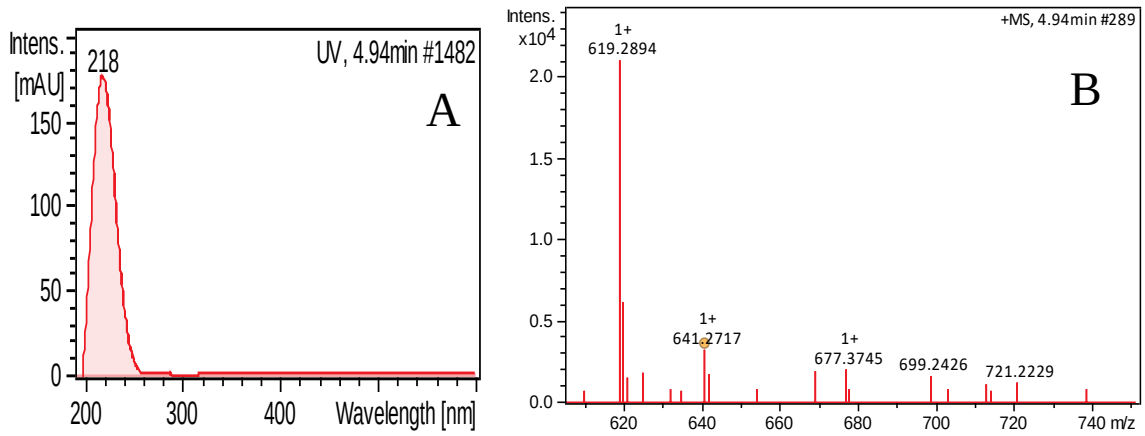


Fig.30. Absorption peaks(A) and MS spectra(B) for $C_{36}H_{46}O_9$ (Not characterized).

Compound sixteen which was detected and not characterized had a molecular formula of $C_{31}H_{36}O_6Na$. This compound recorded a maximum absorption wavelength of 218 nm and 5 signal fragments (m/z) on the MS spectra (483.2754, 505.2580, 518.3272, 527.2393 and 540.3100) with a base peak of 505.2580 and a molecular ion peak of 540.3100 (Fig.31).

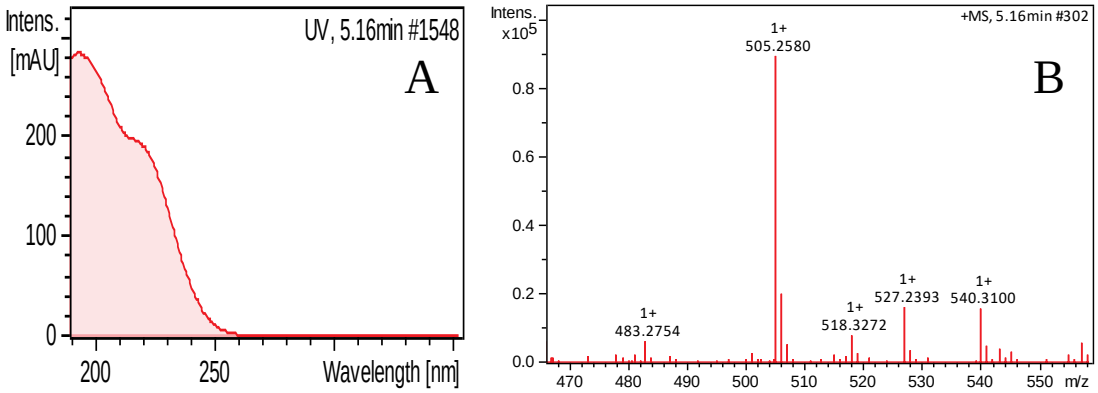


Fig. 31. Absorption peaks (A) and MS spectra(B) for $C_{31}H_{36}O_6Na$ (Not characterized).

III.6.1.2. Flavonoid profiles obtained

Results obtained from the biochemical assay of flavonoids showed that, in all treatments there was a general increase in the flavonoid content after 2 months of growth in both white and red cv *X. sagittifolium* plants. The different flavonoid profiles in the control (0 $t.ha^{-1}$ or 0 $Kg.ha^{-1}$) poultry manure (30$t.ha^{-1}$) and NPK fertilizer (150$kg.ha^{-1}$) treatments were then assessed between 2 and 6 months of growth.

Flavonoid profile results show that there was a general increase in peak intensities between 2 and 6 months of growth for all treatments in both white and red cv of *X. sagittifolium* leaves (Table.23). Analysis of the spectra obtained from the white cv of *X. sagittifolium* leaves after 2 months of planting revealed that the intensities of the first 3 peaks between the retention times of 2.63 min and 3.06 min were more significant in poultry manure treatments (30 $t.ha^{-1}$) and NPK fertilizer treatments (150 $kg.ha^{-1}$) as compared to the control treatments (0 $kg.ha^{-1}$) (Fig. 32 and Table 23). Anyway, the spectra from the red cv of *X. sagittifolium* leaves after 2 months of planting also showed that the intensities of the first 3 peaks between the retention times of 2.63 min and 3.06 min were most significant in the NPK fertilizer treatments (150$kg.ha^{-1}$) than in the control treatments (0 $kg.ha^{-1}$) and the poultry manure treatments (30 $t.ha^{-1}$) (Fig. 33 and Table 23).

Table 23: Peak intensities of Flavonoids in *Xanthosoma sagittifolium* leaves

		Peak intensity(mAU) 2 months after planting			**Peak intensity (mAU) 6 months after planting**		
leaves	Peak (Nº)	Control	Poultry manure	NPK	Control	Poultry manure	NPK
X. sagittifolium (White cv)	1	0.8×10^5	1.1×10^5	1.0×10^5	1.0×10^5	1.1×10^5	0
	2	1.0×10^5	1.4×10^5	1.4×10^5	2.4×10^5	2.4×10^5	0
	3	1.3×10^5	2.1×10^5	1.9×10^5	3.4×10^5	4.4×10^5	3.6×10^5
	4	0.4×10^5	0.55×10^5	0.5×10^5	1.0×10^5	1.5×10^5	1.6×10^5
X. sagittifolium (Red cv)	**1**	0.8×10^5	0.65×10^5	1.5×10^5	1.1×10^5	1.2×10^5	0
	2	1.05×10^5	0.7×10^5	2.5×10^5	2.3×10^5	2.4×10^5	0
	3	1.28×10^5	1.45×10^5	3.8×10^5	3.4×10^5	4.4×10^5	3.68×10^5
	4	0.41×10^5	0.5×10^5	0.6×10^5	0.8×10^5	1.49×10^5	1.6×10^5

Six months after planting the spectra obtained from both white and red cv of *X. sagittifolium* leaves illustrated that the first and second peaks with retention times between 2.63 min and 2.9 min were significantly reduced in intensity with the NPK fertilizer treatments (150 kg.ha^{-1}) as compared to the control treatments (0 kg.ha^{-1}) and the poultry manure treatments (30 t.ha^{-1}). The third peak recorded the most significant intensity values with poultry manure treatments (30t.ha^{-1}) when compared to the control treatments (0 kg.ha^{-1}) and NPK fertilizer treatments (150 kg.ha^{-1}) (Fig.34, Fig.35 and Table 23).

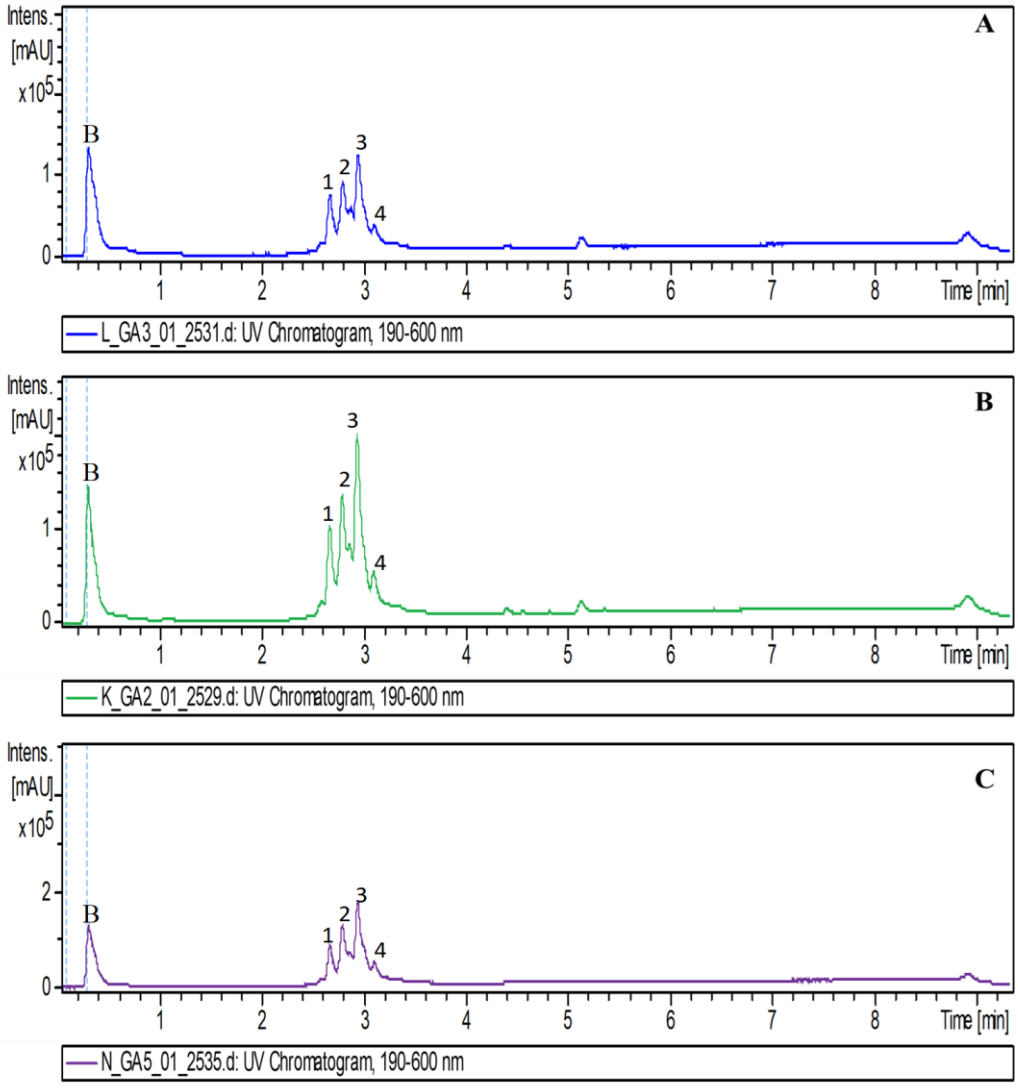


Fig. 32. Flavonoid profiles from white cv leaves two months after planting. Control (A), poultry manure (30t.ha^{-1}) (B) and NPK fertilizer (150kg.ha^{-1}) (C). Blank (B) and flavonoid peaks (1,2,3 and 4).

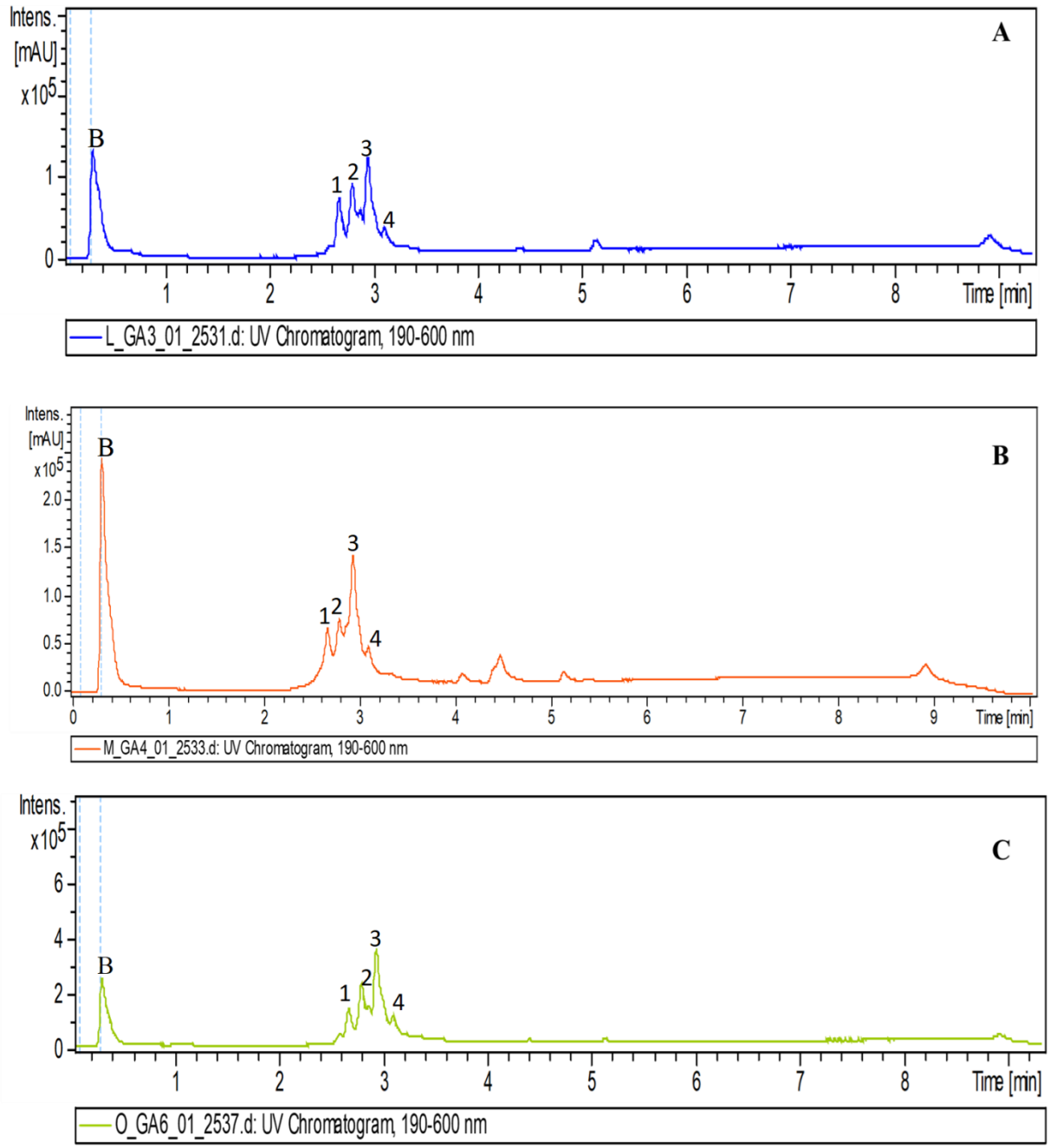


Fig. 33. Flavonoid profiles from red cv leaves 2 months after planting. Control (A), poultry manure (30 $t.ha^{-1}$) (B) and NPK fertilizer (150 $kg.ha^{-1}$) (C). Blank (B) and flavonoid peaks (1,2,3 and 4).

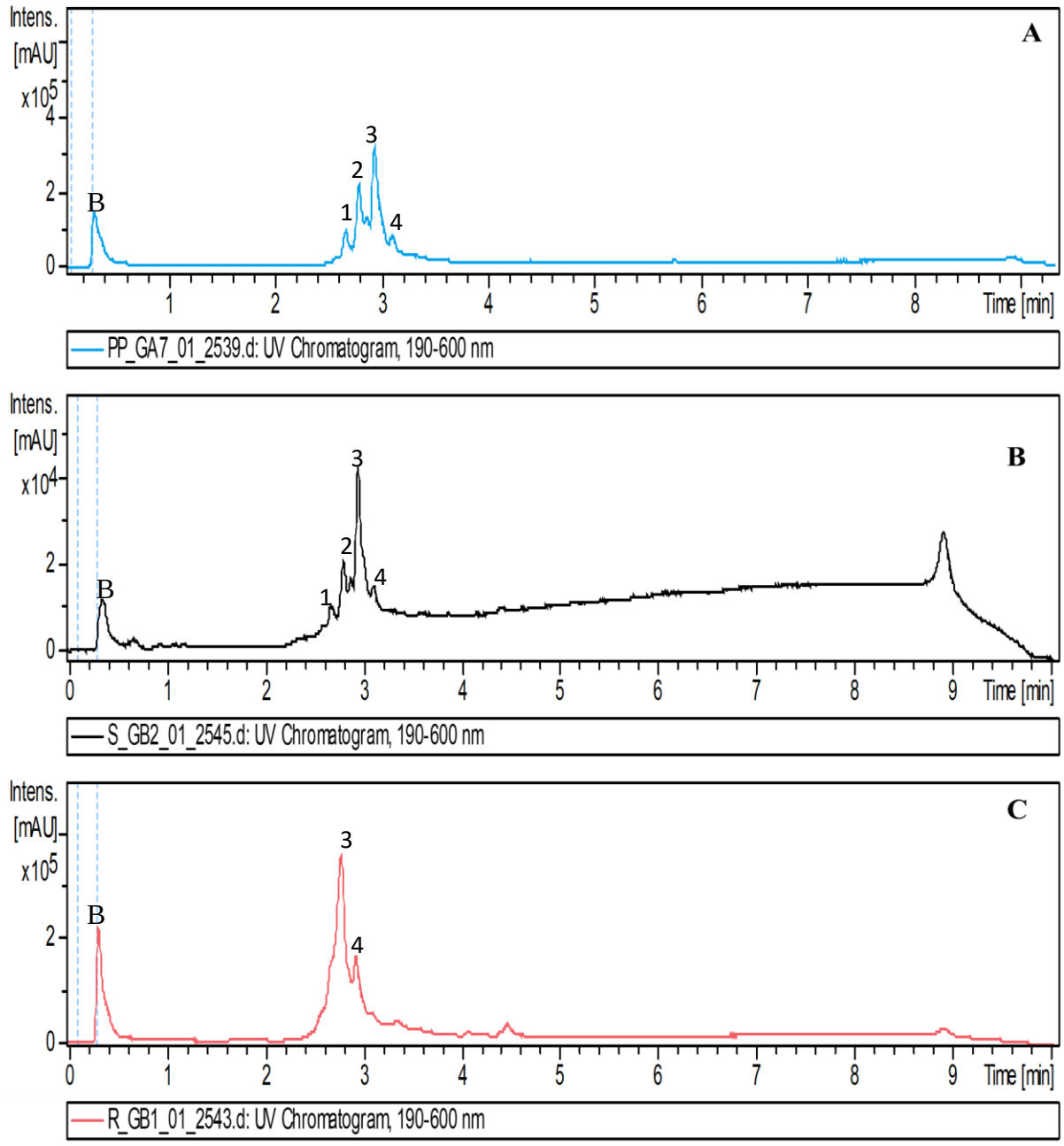


Fig. 34. Flavonoid profiles from white cv leaves 6 months after planting. Control (A), poultry manure (30 t.ha-1) (B) and NPK fertilizer (150 kg.ha-1) (C). Blank (B) and flavonoid peaks (1,2,3 and 4).

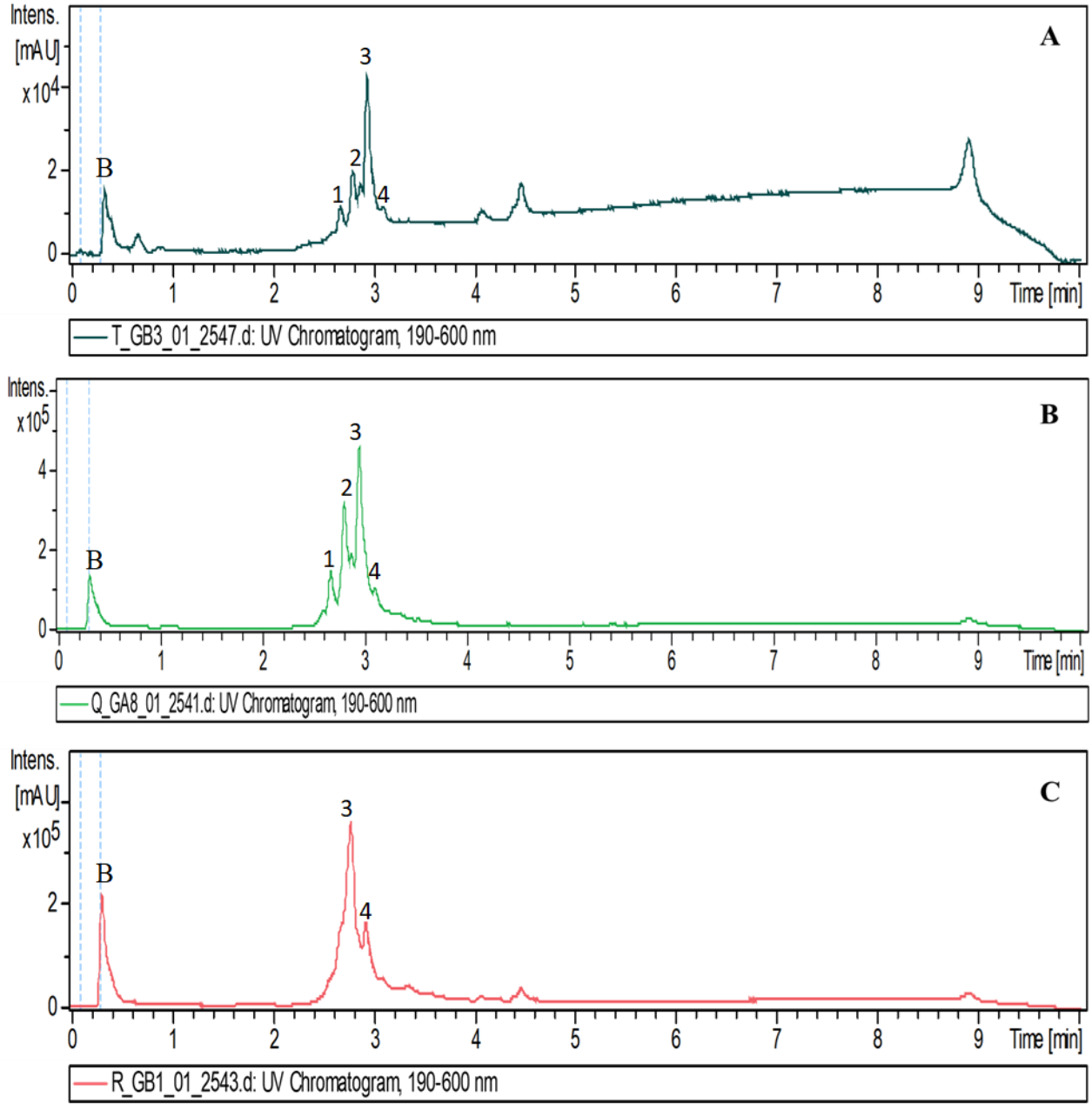


Fig. 35. Flavonoid profiles from red cv leaves 6 months after planting. Control (A), poultry manure (30 $t.ha^{-1}$) (B) and NPK fertilizer (150 $kg.ha^{-1}$) (C). Blank (B) and flavonoid peak (1,2,3and4).

III.6.2. Postulated structures of flavones obtained

All the 8 flavones obtained had the basic structure of apigenin. Glucosyl side units were attached at different positions on basic apigenin structure by condensation reactions giving different compounds. C- glycosidic bonds were formed between the glycosyl units and the apigenin basic structures. Flavones identified on the same peaks were isomers of each other. These compounds were then identified as flavone c-glycosides of apigenin as shown in the figures 36,37,38 and 39 below:

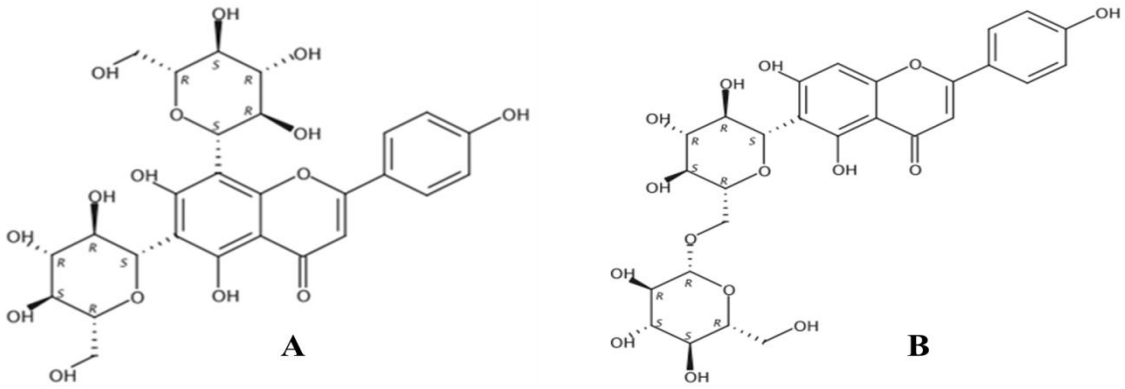


Fig. 36. Flavone metabolite structures with same molecular formula ($C_{27}H_{30}O_{15}$) at 2.36 and 2.72 (min) retention times. 6,8 - Di-C-glucopyranosylapigenin (A) and Isovitexin 6"-O-glucopyranoside (B).

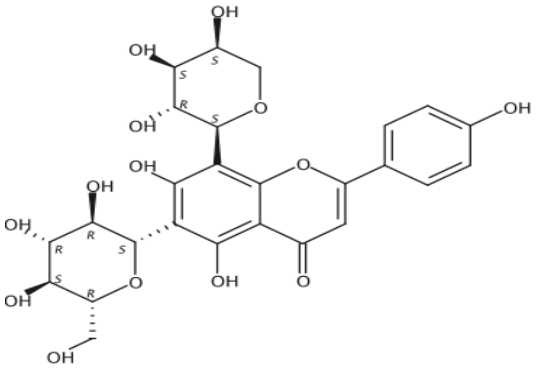


Fig. 37. Isomer structures of three flavone metabolites with same molecular formula ($C_{26}H_{28}O_{14}$) at 2.82, 2.84 and 2.90 (min) retention times. Apigenin 6-C-glucoside 8-C- arabinoside or Apigenin-6-C-pentoside-8-C- hexoside (Isomer 1) or Apigenin-6-C-pentoside-8-C- hexoside (Isomer 2).

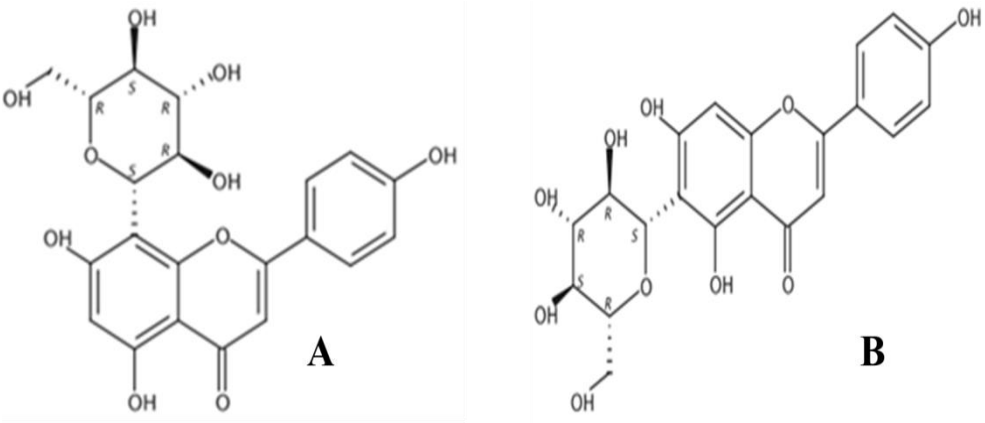


Fig. 38. Flavone metabolite structures with same molecular formula ($C_{21}H_{20}O_{10}$) at 2.97 and 3.06 (min) retention times. 8-C-Glucosylapigenin (vitexin) (A) and 6-C-Glucosylapigenin (Isovitexin) (B).

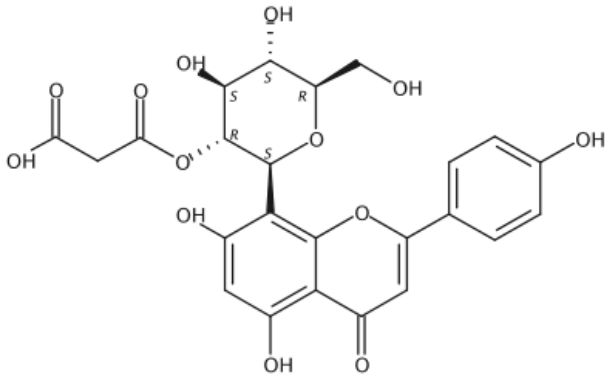


Fig. 39. Flavone metabolite structure of 2"-O-Malonylvitexin with $C_{24}H_{22}O_{13}$ molecular formular at 3.13 (min) retention time.

III.7. Correlation analysis

III.7.1. Correlations between growth and yield parameters

Poultry manure treatments (30t.ha^{-1}) for both white and red cultivar *X. sagittifolium* plants showed positive significant correlations between number of leaves and tuber number per plant, and between number of roots and tuber number per plant (Tables 22 and 23). In white cultivar *X. sagittifolium* plants treated with poultry manure (30t.ha^{-1}) the intensity of correlations between all growth parameters (plant height, number of leaves, leaf area and root number) and all yield parameters (tuber number per plant, tuber weight, tuber length and tuber girth) were positive and high. There was a positive and very significant correlation between plant height and number of leaves (0.977**). The correlations between yield parameters were positive and very significant at 1% level of probability; between tuber number per plant and tuber weight (0.971**), between tuber weight and tuber length (0.957**), between tuber length and tuber girth (0.988**) (Table 24). The correlation intensities between growth and yield parameters in red cultivar *X. sagittifolium* plants treated with poultry manure (30t.ha^{-1}) were positive and high. The correlation between plant height and root number was positive and very significant (0.996**). Correlation intensities between the different yield parameters were non significant (Table 25).

III.7.2. Correlations between growth and biochemical parameters

White cultivar *X. sagittifolium* plants treated with poultry manure (30t.ha^{-1}) depicted significant positive correlations between number of leaves and flavonoid content (0.998*), between root number and flavonoid content (0.999*), and between Protein content and Peroxidase activity (0.998*). The correlations between protein content, peroxidase activity, glucanase activity, polyphenol oxidase activity, flavonoid content on one hand and phenolic content on the the other hand were negative and non significant (Table 26). The red cultivar *X. sagittifolium* plants treated with poultry manure (30t.ha^{-1}) showed non significant correlations between all growth parameters and all biochemical parameters. These treatments also recorded a positive significant correlation between polyphenol oxidase and flavonoid content (0.999*) (Table 27).

III.7.3. Correlations between yield and biochemical parameters

Both white and red cultivar *X. sagittifolium* plants treated with poultry manure ($30t.ha^{-1}$) illustrated non significant correlations between all yield parameters and all biochemical parameter (Tables 28 and 29). White cultivar *X. sagittifolium* plants showed a low negative non significant correlation between tuber girth and polyphenol oxidase (-0.0098^{ns}) (Table 28).

Table 24: Pearson correlation matrix between growth and yield parameters in white cv *X. sagittifolium* plants.

	Number of leaves	**Leaf area**	**Root number**	**Tuber number.plant^{-1}**	**Tuber Weight**	**Tuber Length**	**Tuber girth**
Plant height	0.977	0.929	0.877	0.828	0.72	0.876	0.794
	0.00411	0.0226	0.0506	0.0832	0.17	0.0517	0.109
	**	*	ns	ns	ns	ns	ns
Number of leaves		0.877	0.953	0.881	0.769	0.916	0.852
		0.0507	0.0122	0.0485	0.129	0.0291	0.0666
		ns	*	*	ns	*	ns
Leaf area			0.788	0.872	0.822	0.886	0.837
			0.113	0.0536	0.0874	0.0457	0.0772
			ns	ns	ns	*	ns
Root number				0.919	0.805	0.905	0.88
				0.0273	0.1	0.0345	0.0487
				*	ns	*	*
Tuber number.plant^{-1}					0.971	0.98	0.988
					0.00594	0.00334	0.00158
					**	**	**
Tuber Weight						0.957	0.986
						0.0108	0.00192
						**	**
Tuber Length (cm)							0.988
							0.00166
							**

P< 0.05 (*): correlated significant, P< 0.01 (**): correlated very significant and P< 0.001 (***): correlated and highly significant. ns: non significant.

Table 25: Pearson correlation matrix between growth and yield parameters in red cv *X. sagittifolium* plants

	Number of leaves	**Leaf area**	**Root number**	**Tuber number**	**Tuber weight**	**Tuber length**	**Tuber girth**
Plant height	0.995	0.997	0.996	0.999	0.948	0.815	0.899
	0.0648	0.0516	0.0039	0.0257	0.206	0.394	0.288
	ns	ns	**	*	ns	ns	ns
Number of leaves		1	0.994	0.998	0.911	0.752	0.85
		0.0132	0.0687	0.0391	0.27	0.458	0.353
		*	ns	*	ns	ns	ns
Leaf area			0.996	0.999	0.92	0.765	0.861
			0.0555	0.026	0.257	0.445	0.34
			ns	*	ns	ns	ns
Root number				0.999	0.95	0.818	0.902
				0.0296	0.202	0.39	0.284
				*	ns	ns	ns
Tuber number					0.935	0.791	0.881
					0.231	0.419	0.314
					ns	ns	ns
Tuber weight						0.957	0.992
						0.188	0.0824
						ns	ns
Tuber length							0.986
							0.106
							ns

P< 0.05 (*): correlated significant, P< 0.01 (**): correlated very significant and P< 0.001 (***): correlated and highly significant. ns: non significant.

Table 26: Pearson correlation matrix between growth and biochemical parameters in white cv *X. sagittifolium* plants

	Number of leaves	Leaf area	Root number	Protein content	Peroxidase activity	Glucanase activity	Polyphenol oxidase	Phenolic content	Flavonoid content
Plant height	0.999	0.978	0.99	0.843	0.81	0.933	0.402	0.0588	0.994
	0.0241	0.132	0.0882	0.362	0.399	0.235	0.737	0.963	0.068
	*	ns	ns	ns	ns	ns	ns	ns	ns
Number of leaves		0.97	0.995	0.863	0.832	0.946	0.436	0.0209	0.998
		0.156	0.0641	0.338	0.375	0.211	0.713	0.987	0.0438
		ns	ns	ns	ns	ns	ns	ns	*
Leaf area			0.941	0.713	0.672	0.838	0.204	0.264	0.951
			0.221	0.494	0.531	0.367	0.869	0.83	0.2
			ns	ns	ns	ns	ns	ns	ns
Root number				0.909	0.883	0.974	0.524	-0.0796	0.999
				0.274	0.311	0.147	0.649	0.949	0.0202
				ns	ns	ns	ns	ns	*
Protein content					0.998	0.98	0.832	-0.488	0.895
					0.037	0.127	0.375	0.676	0.294
					*	ns	ns	ns	ns
Peroxidase activity						0.967	0.862	-0.538	0.868
						0.164	0.338	0.639	0.331
						ns	ns	ns	ns
Glucanase activity							0.705	-0.305	0.966
							0.502	0.803	0.167
							ns	ns	ns
Polyphenol oxidase								-0.89	0.497
								0.301	0.669
Phenolic content									-0.0479
									0.969
									ns

P< 0.05 (*): correlated significant, P< 0.01 (**): correlated very significant and P< 0.001 (***): correlated and highly significant. ns: non significant.

Table 27: Pearson correlation matrix between growth and biochemical parameters in red cv *X. sagittifolium* plants

	Number of leaves	Leaf area	Root number	Protein content	Peroxidase activity	Glucanase activity	Polyphenol oxidase	Phenolic content	Flavonoid content
Plant height	0.995	0.997	0.944	0.983	0.667	0.603	0.505	0.348	0.46
	0.0648	0.0516	0.0039	0.119	0.535	0.588	0.663	0.774	0.695
	ns	ns	**	ns	ns	ns	ns	ns	ns
Number of leaves		1	0.994	0.996	0.588	0.681	0.415	0.251	0.368
		0.0132	0.0687	0.0538	0.6	0.523	0.728	0.838	0.76
		*	ns	ns	ns	ns	ns	ns	ns
Leaf area			0.996	0.994	0.605	0.666	0.433	0.271	0.387
			0.0555	0.067	0.587	0.536	0.715	0.825	0.747
			ns	ns	ns	ns	ns	ns	ns
Root number				0.982	0.672	0.598	0.51	0.354	0.466
				0.123	0.531	0.592	0.659	0.77	0.692
				ns	ns	ns	ns	ns	ns
Protein content					0.518	0.741	0.336	0.168	0.288
					0.654	0.469	0.782	0.892	0.814
					ns	ns	ns	ns	ns
Peroxidase activity						-0.192	0.98	0.931	0.968
						0.877	0.128	0.239	0.16
						ns	ns	ns	ns
Glucanase activity							-0.384	-0.538	-0.43
							0.749	0.639	0.717
							ns	ns	ns
Polyphenol oxidase								0.985	0.999
								0.111	0.0325
								ns	*
Phenolic content									0.992
									0.0782
									ns

P< 0.05 (*): correlated significant, P< 0.01 (**): correlated very significant and P< 0.001 (***): correlated and highly significant. ns: non significant

Table 28: Pearson correlation matrix between yield and biochemical parameters in white cv *X. sagittifolium* plants

	Tuber length	**Tuber girth**	**Tuber protein content**	**Peroxidase activity**	**Glucanase activity**	**Polyphenol oxidase**	**Phenolic content**	**Flavonoid content**
Tuber weight	0.987 0.104 ns	0.995 0.0629 ns	0.462 0.694 ns	0.41 0.731 ns	0.629 0.567 ns	-0.108 0.931 ns	0.549 0.63 ns	0.809 0.4 ns
Tuber length		0.998 0.0416 *	0.601 0.59 ns	0.553 0.627 ns	0.747 0.463 ns	0.0554 0.965 ns	0.405 0.735 ns	0.894 0.296 ns
Tuber girth			0.547 0.631 ns	0.498 0.668 ns	0.702 0.504 ns	-0.00982 0.994 ns	0.464 0.693 ns	0.863 0.337 ns
Protein content				0.998 0.037 *	0.98 0.127 ns	0.832 0.375 ns	-0.488 0.676 ns	0.895 0.294 ns
Peroxidase activity					0.967 0.164 ns	0.862 0.338 ns	-0.538 0.639 ns	0.868 0.331 ns
Glucanase activity						0.705 0.502 ns	-0.305 0.803 ns	0.966 0.167 ns
Polyphenol oxidase							-0.89 0.301 ns	0.497 0.669 ns
Phenolic content								-0.0479 0.969 ns

P< 0.05 (*): correlated significant, P< 0.01 (**): correlated very significant and P< 0.001 (***): correlated and highly significant. ns: non significant.

Table 29: Pearson correlation matrix between yield and biochemical parameters in red cv *X. sagittifolium* plants

	Tuber length	**Tuber girth**	**Protein content**	**Peroxidase activity**	**Glucanase activity**	**Polyphenol oxidase**	**Phenolic content**	**Flavonoid content**
Tuber weight	0.957 0.188 ns	0.992 0.0824 ns	0.873 0.324 ns	0.869 0.33 ns	0.319 0.793 ns	0.753 0.457 ns	0.628 0.568 ns	0.718 0.49 ns
Tuber length		0.986 0.106 ns	0.693 0.512 ns	0.975 0.141 ns	0.0292 0.981 ns	0.912 0.269 ns	0.827 0.38 ns	0.89 0.302 ns
Tuber girth			0.803 0.407 ns	0.926 0.247 ns	0.194 0.876 ns	0.831 0.375 ns	0.723 0.486 ns	0.802 0.408 ns
Protein content				0.518 0.654 ns	0.741 0.469 ns	0.336 0.782 ns	0.168 0.892 ns	0.288 0.814 ns
Peroxidase activity					-0.192 0.877 ns	0.98 0.128 ns	0.931 0.239 ns	0.968 0.16 ns
Glucanase activity						-0.384 0.749 ns	-0.538 0.639 ns	-0.43 0.717 ns
Polyphenol oxidase							0.985 0.111 ns	0.999 0.0325 *
Phenolic content								0.992 0.0782 ns

P< 0.05 (*): correlated significant, P< 0.01 (**): correlated very significant and P< 0.001 (***): correlated and highly significant. ns: non significant.

III.7.4. Principal component analysis (PCA) between growth parameters and some secondary metabolites

After 6 months of culture the correlation circle in the white cv *X. sagittifolium* plants show an explanation for principal component 1 axis (F1) of 51.02% while principal component axis 2 (F2) explained 27.29% of variance. The combined effect of both principal component axes 1 and 2 explained 78.30% of the total variance. In red cv *X. sagittifolium* plants, principal component 1 axis (F1) recorded 47.59% while principal component 2 axis (F2) had 32.94% of variance, with a combined total effect for both axes of 80.52% (Fig.40).

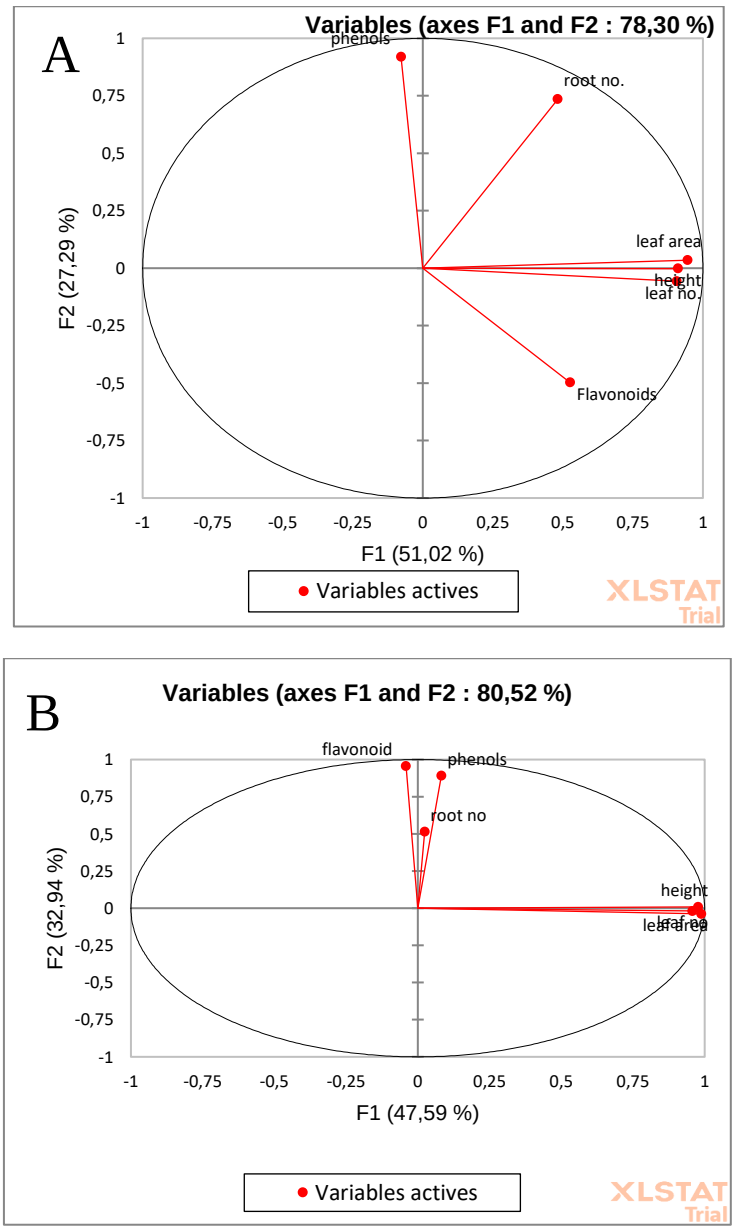


Fig. 40. Correlation circles between growth parameters (average height, leaf number, root number, leaf area) phenol and flavonoid content in (A) white cv and (B) red cv *X. sagittifolium* plants.

The factorial diagrams of principal component analysis (PCA) in both white cv and red cv *X. sagittifolium* plants confirmed a positive correlation between average plant height, leaf number and leaf area in samples treated with 30t ha^{-1} of poultry manure (PM2). No correlations were observed between average plant height, leaf number, leaf area and phenol content for both cultivars. In red cv plants NPK2(150kg.ha^{-1}) and PM2 (30t.ha^{-1}) samples are closely associated to each other (Fig. 41).

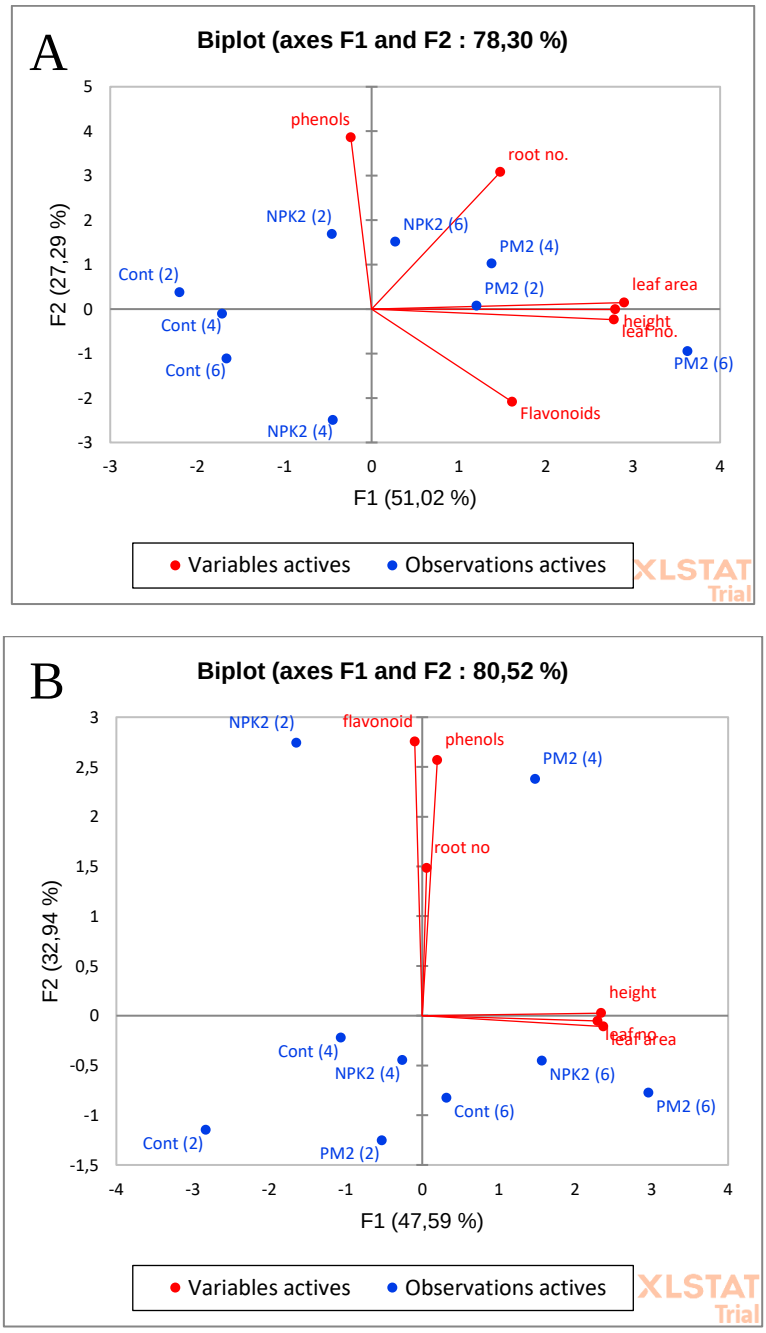


Fig. 41. Principal component analysis factorial diagrams of data relative to the effect of different fertilizer treatments (poultry manure and NPK fertilizer) on some agromorphological and some secondary metabolites in (A) white cv and (B) red cv *X. sagittifolium* plants.

Key: Cont= control, PM1= poultry manure (20t.ha^{-1}), PM2= poultry manure (30t.ha^{-1}), NPK1= NPK1 fertilizer (120kg.ha^{-1}), NPK2= NPK fertilizer (150kg.ha^{-1})

III.7.5. Principal component analysis between parameters of growth, yield and soil cation exchange capacity

At harvest, the correlation circle in the white cv *X. sagittifolium* plants indicate an explanation for principal component 1 axis (F1) of 85.56% while principal component axis 2 (F2) explained 8.25% of variance. The combined effect of both principal component axes 1 and 2 explained 93.80% of the total variance. In red cv *X. sagittifolium* plants, principal component 1 axis (F1) recorded 79.02% while principal component 2 axis (F2) had 13.70% of variance, with a combined total effect for both axes of 92.72% (Fig.42).

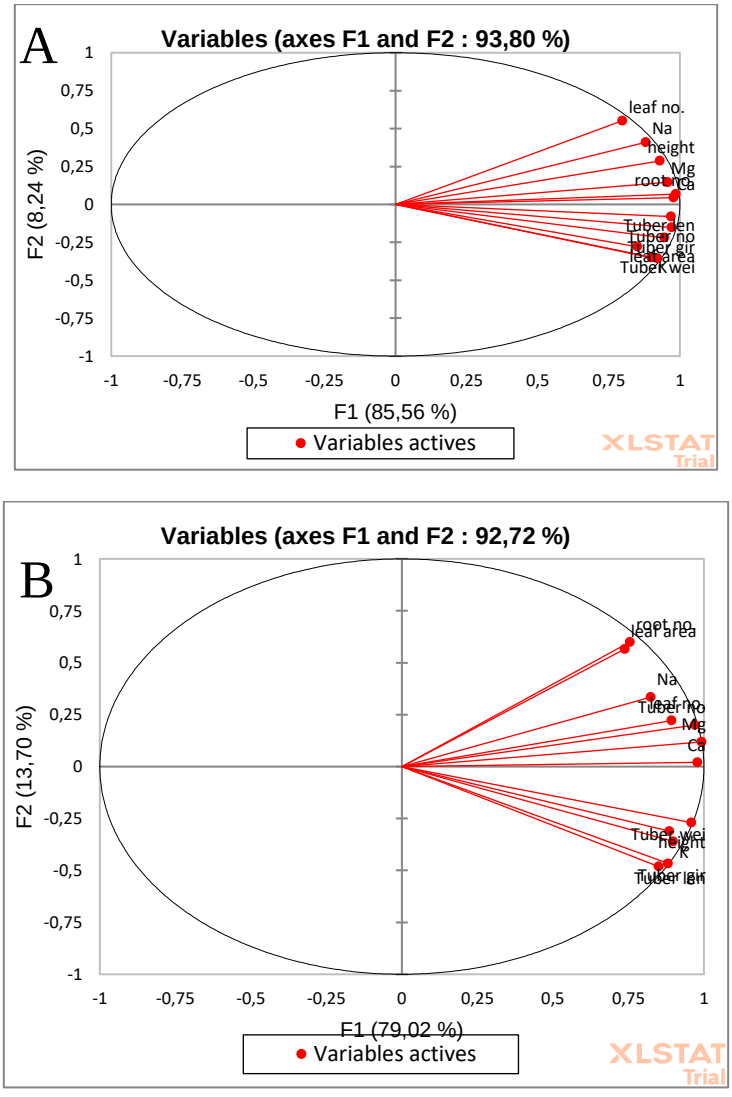


Fig. 42. Correlation circles between parameters of growth (average height, leaf number, root number, leaf area), yield (average tuber number, tuber weight, tuber length and tuber girth) and soil exchangeable cations (K^+, Mg^{++}, Na^+ an Ca^{++}) in (A) white cv and (B) red cv *X. sagittifolium* plants.

In both white and red cv *X. sagittifolium* plants, positive correlation exists between parameters of growth, yield and soil exchangeable bases as indicated in factorial diagrams of principal component analysis (PCA). High positive correlations between sodium and magnesium exchangeable bases with average leaf number were observed while exchangeable potassium bases were highly correlated with yield parameters in both cultivars. In white cv plants PM1 (20t.ha^{-1}) and NPK2 (150kg.ha^{-1}) samples are closely associated to each other while in red cv plants, control and NPK1(120kg.ha^{-1}) samples show a close association (Fig. 43).

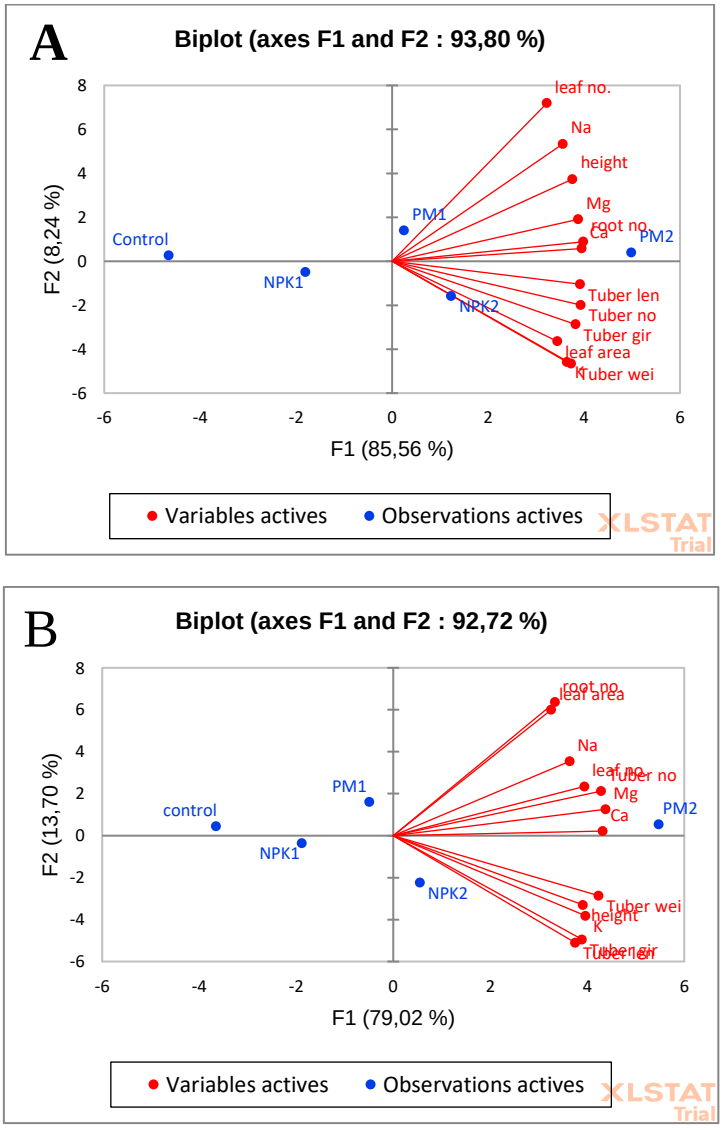


Fig. 43. Principal component analysis factorial diagrams of data relative to the effect of different fertilizer treatments (poultry manure and NPK fertilizer) on some agromorphological parameters and some soil exchangeable cations in (A) white cv and (B) red cv *X. sagittifolium* plants.

Key: Cont= control, PM1= poultry manure (20t.ha^{-1}), PM2= poultry manure (30t.ha^{-1}), NPK1= NPK1 fertilizer (120kg.ha^{-1}), NPK2= NPK fertilizer (150kg.ha^{-1}), Na= sodium, Mg= magnesium, Ca= calcium, K= potassium

CHAPTER IV: DISCUSSION, CONCLUSION, PERSPECTIVES

IV.1. DISCUSSION

The objective of this pioneering field trial of *Xanthosoma sagittifolium* (white and red cultivars) minitubers as seeds was to evaluate their performance in response to different treatments of poultry manure and NPK fertilizers. From the results obtained poultry manure and NPK fertlizer treatments significantly influenced growth, development and tuber yield in white and red cv *X. sagittifolium* plants.

Soil fertility is very critical for optimum production of crops. Results from physical analyses of different treatment plots before planting and after harvest showed that the soil textural was clay loam before planting and remained clay loam after harvest for all treatment plots. This implies that poultry manure and NPK fertilizer treatments did not significantly influence soil texture. Poultry manure treatments significantly improved soil moisture content as compared to the NPK fertilizer and control treatments. The soil organic content of poultry manure treatments was higher than that of NPK fertilizer treatments and could account for the increase in the moisture holding capacity of soil particles. Increased levels of organic matter led to greater pore space with the immediate result that water infiltrated more readily and could be held in the soil (Jehangir *et al.,* 2017). Organic matter has been reported to stabilize soil structure thereby reducing bulk density, increasing porosity and water content (Ihenacho *et al.,* 2015). Agbede *et al.* (2017) studied the impact of poultry manure and NPK fertilizer on soil physical properties and growth and yield of carrot (*Daucus carota* L.). They showed that poultry manure reduced soil bulk density and temperature, and improved total porosity and moisture content compared to the NPK fertilizer and the control. In addition, Khalid *et al.* (2014) conducted a field trial on the effects of poultry manure and NPK Fertilizer on physical properties of a sandy soil in Ghana and revealed that poultry manure decreased the dry bulk density, increased the total porosity and increased the moisture content but NPK fertilizer did not show any significant improvement in any of these parameters.

Our results from chemical analyses of the soil show that before planting the soil pH was slightly acidic. After harvest there was no significant pH difference between the control and NPK fertilizer treatments but the poultry manure treatments significantly increased soil pH. Poultry manure contains basic cations which give it the ability to increase soil pH and decrease soil acidity. Poultry manure contains more plant nutrients than all other organic

manures. Decomposed poultry manure stimulates microbial activities which contribute to soil fertility restoration. Effective microorganisms will accelerate the breakdown of organic matter from the manure to humus (Hu *et al.,* 2011). Together with the beneficial effect of chicken manure as the source of nutrients and high cation exchange capacity (CEC), effective microorganisms will be able to increase crop yields and improve crop quality. It will also increase the population of beneficial microorganisms in the soil (Zhen *et al.*, 2014).

The total organic matter and carbon content values in the control and NPK fertilizer treatment plots after harvest did not differ significantly from those obtained in the soil samples before planting, meanwhile the poultry manure treatment plots showed a very significant increase in total organic matter and carbon as compared to the other treatments. This is evidenced by the fact that the chemical analysis of the poultry manure used had high percentages of organic matter and carbon contents. Gülsüm *et al.* (2019) also showed that poultry manure applications improved soil organic matter content.

Poultry manure and NPK fertilizer treatments significantly improved the total nitrogen available phosphorus contents compared to the control treatments. Exchangeable Calcium, Magnesium, Potassium and Sodium had more significant values in poultry manure treatments compared to the control treatments. Agbede *et al*. (2017) showed that poultry manure relatively increases soil Nitrogen, Phosphorous, Potassium, Calcium, Magnesium, and organic Carbon. Nitrogen, Phosphorous and Potassium are primary nutrients which are needed by plants in fairly large quantities for their growth and development. Poultry manure contains all 13 of the essential plant nutrients that are used by plants. These include nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), manganese (Mn), copper (Cu), zinc (Zn), chlorine (Cl), boron (B), iron (Fe), and molybdenum (Mo). Using poultry manure as a fertilizer for crops may provide a portion, or all of the plant requirements. The amount of nutrients provided depends on the nutrient content of the manure and the amount of manure applied (Chastain *et al.,* 2001).

Cation exchange capacity (CEC) is a useful indicator of soil fertility because it shows the soil's ability to supply important plant nutrients: calcium, magnesium, potassium and sodium (Hazleton and Murphy 2007). The cation exchange capacities (CEC) was most significant in poultry manure treatments than the other treatments after harvest. Cations which are bound on the soil’s surface can exchange places with cations found in the soil’s solution, making them available to plants. The higher the cation exchange capacity, the higher the

negative charge of the soil and the more cations that can be held, thereby increasing the holding capacity of soil nutrients. These nutrients can then be slowly released to plants. Soil pH is important for CEC because as pH increases (becomes less acid), the number of negative charges on the colloids increase, thereby increasing CEC. CEC varies according to the type of soil (Hazleton and Murphy 2007). Clay and organic matter have negative charges which attract positively charged cations and hold them on the surface of soil particles. Humus, the end product of decomposed organic matter, has the highest CEC value because organic matter colloids have large quantities of negative charges. Clay has a great capacity to attract and hold cations because of its chemical structure. Sand has no capacity to exchange cations because it has no electrical charge (Hazleton and Murphy 2007). Since the poultry manure treatments significantly improved soil organic content and increased soil pH, this could also account for the increase in cation exchange capacities observed in these treatments as compared to the other treatments used.

The growth and yield performance for the white cultivar cocoyam plants were significantly higher than those for the red cultivar cocoyam plants. These results are concordant with those obtained by Nzietchueng (1985) who evaluated the production problems faced by different cocoyam (*Xanthosoma*) cultivars and showed that the white cultivar was more productive than the red cultivar. In both white and red cv *X. sagittifolium* plants a significant increase in average plant height was observed in all treatments during the first 6 months of growth followed by a significant decrease in average height after 6 months of growth. The most significant increase in average height was recorded by the poultry manure treatments (30 $t.ha^{-1}$) as compared to the control treatments. These results show that there was a stimulation in plant growth hormones during the first 6 months of growth. Poultry manure treatments probably influenced water and mineral nutrition which stimulated plant growth. Agyei and Bayor (2017) demonstrated that poultry manure treatments of 40 $t.ha^{-1}$ and 70 $t.ha^{-1}$ significantly increased the plant heights of carrot (*Daucus carota)* plants. Ihenacho *et al*. (2015) also depicted that poultry manure applications of 8 $t.ha^{-1}$ increased plant height significantly in Turmeric (*Curcuma Longa* L) plants. It could be suggested that the decrease in average plant height after 6 months was due to senescence and regeneration of leaves, agreeing with Djeuani *et al.* (2017) who illustrated that after after 120 days the average heights of acclimatized X. *sagittifolium* plants reduced with time. In white cv *X. sagittifolium* plants there was no significant difference in the average number of leaves per plant after 2 months of growth but after 6 months of growth poultry manure treatments (30 $t.ha^{-1}$) had a

more significant average leaf number as compared to the control treatments. This could suggest that poultry manure treatments provided water and mineral nutrition which favoured growth and development of leaves. All treatments showed a significant decrease in the average number of leaves after 6 months of growth. In red cv *X. sagittifolium* plants there was no significant difference in the average number of leaves for all treatments during growth. Agyei and Bayor (2017) in the study of the effects of poultry manure and nitrogen, phosphorus, and potassium (15:15:15) soil amendment on growth and yield of carrot (*Daucus carota*) also showed that there was no significant difference in the average number of leaves among all the different treatments applied. After 6 months of growth there was a significant decrease in the average leaf number. Similarly, Djeuani *et al.* (2017) observed a significant decrease in the average leaf number after 120 days of growth in *X. sagittifolium* plants during minituber formation.

Both white and red cv *X. sagittifolium* plants showed a significant increase in average leaf area in all during the first 6 months of growth followed by a significant decrease in average leaf area after 6 months of growth in all treatments. The most significant increase in average leaf area was recorded by the poultry manure treatments (30 $t.ha^{-1}$) as compared to the control treatments. These results show that there was a stimulation in plant growth hormones and photosynthetic factors during the first 6 months of growth. Poultry manure treatments probably influenced more water and mineral nutrition which stimulated more photosynthetic activities in the leaf. Okoli and Nweke (2015) who worked on the effect of different rates of poultry manure on growth and yield of Amarathus (*Amaranthus cruentus*) showed an increase in the number of leaves and leaf area during growth. The general decrease in the average leaf surface area recorded in both white and red cv *X. sagittifolium* plants in all treatments after 8 months of growth could be attributed to a decrease in photosynthetic activity leading to the maturation of tubers. Djeuani *et al*. (2017) observed that the leaves produced after 120 day of growth in acclimatized *X. sagittifolium* vitro plants were smaller in size than those produced before 120 days.

Average root number decreased progressively after 2 months of growth in white cv *X. sagittifolium* plants for the control and poultry manure treatments. This decrease can be attributed to renewal of roots. Djeuani *et al.* (2017) showed a progressive decrease in the average number of roots after 120 days for all treatments of mycorrhizae in *X. sagittifolium* plants during minituberizaton. The NPK fertilizer treatments showed a very significant

decrease in average root number between 2 months and 4 months of growth followed by a significant increase at 6 months of growth and a decrease at 8 months of growth. NPK fertilizer treatments may have increased soil salinity and soil osmotic pressure causing the roots in these treatments to loss more water and wither leading to the significant decrease in average root number observed. Machodo and Serralheiro (2017) depicted that a high salt concentration in soil solution reduces the ability of plants to acquire water, which is referred to as the osmotic or water-deficit effect of salinity. The osmotic effect of salinity induces metabolic changes in the plant identical to those caused by water stress-induced "wilting" (Munns *et al.*, 2002).

In red cv *X. sagittifolium* plants, all the poultry manure treatments and the NPK fertilizer treatment (150kg.ha^{-1}) showed a decrease in average root number after 4 months of growth while the control and NPK fertilizer treatment (120 kg.ha^{-1}) recorded a decrease in average root number after 6 months of growth. This may be attributed to the fact that during the maturation of tubers less nutrients are required from the soil.

At harvest poultry manure treatments (30t.ha^{-1}) showed the most significant yield parameters as compared to the control treatments in both white and red cv treatments. Poultry manure (30t.ha^{-1}) white cv *X. sagittifolium* tubers recorded mean tuber number per plant (8), mean tuber weight (250g), mean tuber length (14cm) mean tuber girth (18cm) while control white cv *X. sagittifolium* tubers had recorded mean tuber number per plant (2), mean tuber weight (20g), mean tuber length (6cm) and mean tuber girth (10.5cm). Poultry manure (30t. ha^{1}) red cv *X. sagittifolium* tubers recorded mean tuber number per plant (5.7), mean tuber weight (124.7g), mean tuber length (8cm) mean tuber girth (14.5cm) while control red cv *X. sagittifolium* tubers had mean tuber number per plant (2.3), mean tuber weight (22g), mean tuber length (3cm) and mean tuber girth (6cm). The significant weight values observed could be attributed to the presence of a carbon and nitrogen sources (C/N ratio). Tubers serve as storage sinks or reserve organs for sugars (Du jardin, 1994). The improvement in the soil organic carbon by poultry manure treatments could also account for the increase in size and weight of tubers obtained from these treatments. Tsafack (2010) and Djeuani *et al.* (2014) showed that the carbon source had a positive impact on the induction of tuberization in *X. sagittifolium.* With adequate N, at full genetic potential, more C-assimilation per unit N would increase biomass (Lawlor, 2002)

These results illustrated a better yield parameter in white cv *X. sagittifolium* plants than in red cv *X. sagittifolium* plants for all treatments confirming the results obtained by Nzietcheung (1985) that white cv *X. sagittifolium* plants are more productive than red cv *X. sagittifolium* plants. As observed from the above results, it was evident that poultry manure (30 $t.ha^{-1}$) treatments significantly produced the highest means of traits assessed, while the control treatments of no poultry manure or no NPK fertilizer applied significantly produced the lowest means of the same traits over a 9 month growth and yield period in both cultivars. 150 kg ha^{-1} NPK fertilizer treatments expressed the most significant means of traits among all the NPK fertilizer treatments in both cultivars. Similar observations were earlier reported by Hamma *et al.* (2014) when they evaluated the performance of cocoyam (*Colocasia esculentus.* L) under the influence of organic and inorganic manure in Samaru, Zaria, Nigeria. They observed that poultry manure ($10t.ha^{-1}$) produced the most significant means of growth and yield parameters among treatments of no manure (control), goat manure ($10t.ha^{-1}$) and cow manure ($10t.ha^{-1}$). They also showed that $150kg.ha^{-1}$ NPK fertilizer treatments produced the most significant means of growth and yield traits among no NPK fertilizer (control), 90 kg $NPK.ha^{-1}$ and120 kg $NPK.ha^{-1}$ fertilizer treatments.

Our results depicted better crop performance for parameters measured with poultry manure ($30t.ha^{-1}$) compared to NPK fertilizer amendments for both white cv and red cv. This could be attributed to the fact that poultry manure soil amendments had a more favourable influence on soil pH, and soil organic content by increasing them and recorded higher cation exchange capacity values than all other treatments. Gülsüm *et al*. (2019) also showed that poutry manure applications improved soil organic matter content, exchangeable cations, cation exchange capacity, and percent base saturation thereby enhancing yield of sweet basil (*Ocimum basilicum* L.). Therefore, poultry manure treatment plots retained more nutrients in the soil than the other treatments and slowly released these nutrients to the plants with reduced leaching losses of nutrients throughout the growth period (Uwah *et al.*, 2011). The chemical composition of the different treatment plots after harvest showed that those treated with poultry manure ($30t.ha^{-1}$) had a higher N, K and organic carbon content which could also account for better growth and yield performance. These results agree with those obtained by Uwah *et al*. (2011), who evaluated the effect of organic and mineral fertilizers on growth and yield of taro (*Colocasia esculenta* (L.) Schott and showed that when the N, P, K and Ca contents of poultry manure increased on a site, it led to superior crop performance.

Concordant results have also been obtained by Karamat *et al.* (2019) who showed that poultry litter had a significant increase in grain yield and N,P,K uptake by corn (*Zea mays*).

The nutritional analysis of harvested tubers showed that red cv *X. sagittifolium* tubers (cormels) had a more significant crude protein content than the white cv *X. sagittifolium* tubers for all treatments. Poultry manure treated red cv tubers had the most significant values (18.14%), suggesting that the high C/N ratio of the poultry manure applied favoured the synthesis of proteins in cormels. Ezeocha *et al.,* (2014) also evaluated poultry manure application rates on the nutrient composition of *Dioscorea bulbifera* (Aerial yam) and demonstrated a significant increase in the crude protein content (6.28%) in harvested tubers with poultry manure applications of 3t ha^{-1}.The results obtained from the analysis of the macro mineral content of harvested tubers showed that calcium (Ca), magnesium (Mg), sodium (Na), phosphorus (P) and nitrogen(N) were more significant in red cv *X. sagittifolium* tubers than in the white cv *X. sagittifolium* tuber for all treatments. The white cv *X. sagittifolium* tubers recorded a more significant potassium (K) content than the red cv *X. sagittifolium* tubers for all treatments. Poultry manure treated red cv tubers had more significant nitrogen and sodium contents as compared to the control tubers. These results are contrary to those obtained by Ezeocha *et al.* (2014) who evaluated poultry manure application rates on the nutrient composition of *Dioscorea bulbifera* (Aerial yam) and showed that phosphorus, calcium and magnesium contents were significantly increased by poultry manure application. Mwenye *et al.* (2011) also studied mineral composition of Malawian cocoyam (*Colocasia esculenta* and *Xanthosoma sagittifolium*) genotypes and demonstrated that Malawian cocoyam are also good sources of the essential mineral nutrients in addition to cereal crops.

Poultry manure and NPK fertilizer treatments induced biochemical responses in the leaves of *X. sagittifolium* plants during growth. Proteins are large molecular weight macromolecules made up of amino acids linked by peptide bonds (Green *et al.,* 2002). Proteins play a direct role in the growth and development of plants and could therefore be regarded as primary metabolites. The results show that the total soluble protein content in the leaves of white cv *X. sagittifolium* plants decreased with time in the control and NPK ($150kg.ha^{-1}$). Djeuani *et al.* (2017) who worked on the effect of arbuscuar mycorrhizal fungi on mintuberization in *X. sagittifolium* plants obtained a decrease in total soluble leaf protein content over time especially during maturation of *X. sagittifolium* minitubers. Borgmann *et al.*

(1994) also demonstrated that the total protein content decreased during the maturation of microtubers in irish potatoes. But contrary results were obtained in the poultry manure ($30t.ha^{-1}$) treated white cv *X. sagittifolium* plants which had a non significant increase in total soluble protein content during the first 6 months growth. Since proteins play an important role in the growth and repair of plant cells, this could suggest a positive correlation between protein content and the different growth parameters assessed during growth of plants treated with Poultry manure ($30t.ha^{-1}$). In red cv *X. sagittifolium* plants the control treatments indicated no significant difference in leaf total soluble protein content during 6months of growth, there was a significant decrease in leaf total soluble protein content between 2 and 4 months of growth followed by a significant increase at 6 months of growth in poultry manure treatments ($30t.ha^{-1}$). The NPK fertilizer ($150kg.ha^{-1}$) treatments recorded a significant decrease in total soluble protein content between 4 and 6 months of growth.

These variations in total soluble protein content could be due to differences in the available mineral nutritional factors like nitrogen and carbon (C/N ratio) in each treatment plot, which affects protein formation in leaves. The C/N ratio value of the poultry manure used was significant. Nitrogen (N) is an essential nutrient that plants require for the synthesis of amino acids, proteins, and many other important metabolites. Consequently, the amount of N that is assimilated and distributed from roots to source leaves and finally to developing sinks, like fruits and seeds, has significant consequences for plant metabolism and growth. Importantly, nitrogen and carbon metabolism are highly interrelated (Palenchar *et al.,* 2004; Krapp and Truong 2005; Nunes-Nesi *et al.,* 2010). Photosynthesis and subsequent respiration provide the C skeletons and energy required for the synthesis of amino acids (Lewis *et al.,* 2000; Nunes-Nesi *et al.,* 2010), whereas the majority of leaf N is present in proteins essential for C assimilation and metabolism (Evans and Poorter, 2012; Hikosaka and Terashima, 1996). In fact, ribulose bisphosphate carboylase (Rubisco), the primary enzyme in C- fixation, alone can account for up to 30% of the total N and 60% of the soluble proteins in mature source leaves (Makino and Osmond, 1991; Osaki *et al*., 1993; Warren *et al.,* 2010). Pernollet *et al.* (1986) worked on the relationship between photosynthesis and protein synthesis in maize and illustrated inter conversions of the photoassimilated carbon in the ear leaf and in the intermediary organs to synthesize the seed storage proteins and starch. Leaves contain the complex biochemical photosynthetic machinery capable of capturing light and using the energy for the reductive assimilation of carbon dioxide (CO_2) and nitrate ions (NO_3) with the formation of carbohydrates and amino acids, respectively (Foyer *et al.,* 2012; Lawlor, 2002).

The interactions between carbon dioxide (CO_2) and nitrate (NO_3) assimilation and their dynamics are of key importance for crop production. In particular, an adequate supply of NO_3, its assimilation to amino acids (for which photo-synthesized carbon compounds are required) and their availability for protein synthesis, are essential for metabolism. An adequate supply of NO_3 stimulates leaf growth and photosynthesis (Lawlor, 2002).

The results obtained from the assay of peroxidase activity, showed that there was a general increase in peroxidase activity in all treatments for both white and red cv *X. sagittifolium* leaves during the first 6 months of growth. These results are concordant with those obtained by Djeuani (2018) who studied the evolution of some biochemical parameters during mintuberization in *X. sagittifolium* plants and demonstrated that Guaicol peroxidasic activities evaluated in leaves increase with time. This increase could be attributed to a rise in both abiotic or biotic stress or an initiation of organogenesis. Tsafack (2010) showed that during microtuber formation in *X. sagittifolium* the initiation of micro tubers is accompanied by an activation of Guaicol peroxidase activity with the appearance of new isoperoxidases. Similarly, Kamegne and Omokolo (2002) showed that peroxidases are involved in organogenesis in *X. sagittifolium*. Baaziz *et al.* (2006) and Mas Athira *et al.* (2019) also illustrated that generally, in case of a wound, microbial infection or any other unfavourable condition, new isoperoxidases appear. Altaey and Majid (2018) studied the effects of water salinity and organic fertilization in lettuce (*Lactuca sativa* L.) on contents of antioxidant enzymes and showed that an increase in water salinity (abiotic stress), increases peroxidase content in leaves.

β -1,3-glucanases activity increased significantly during growth in the poultry manure ($30t.ha^{-1}$) treatments of white and red cv *X. sagittifolium* leaves. The white and red cv control plants together with the NPK fertilizer ($150kg.ha^{-1}$) white cv plants showed a significant increase in β-1,3- glucanases activity after 4 months of growth followed by a decrease at 6 months of growth. The most significant levels of β-1,3- glucanases activities in both cultivars was observed in *X. sagittifolium* plants treated with poultry manure ($30t.ha^{-1}$) at 6 months of growth. This suggests the roles of glucanases in plant stress relief, agreeing with Vaiyapuri *et al.* (2012) who evidenced that β-1,3-glucanases play key roles in cell division, impeding cell to cell virus movements in plants by regulating callose turnover at plasmodesmata, and withstanding abiotic stress. Wang *et al.* (2019) also revealed the involvement of glucanases in fungal growth inhibition mechanisms by rhizobacteria (*Paenibacillus jamilae* HS-26).

Polyphenol oxidase (PPO) assay revealed variations in content for both white and red cv *X. sagittifolium* plants. The white cv control treatments showed a significant increase in polyphenol oxidase during growth while red cv control treatments showed no significant difference in PPO content during growth. Poultry manure treatments ($30t.ha^{-1}$) in both white and red cv plants showed a significant increase in PPO activity at 4 months of growth followed by a decrease at 6 months of growth. The NPK fertilizer ($150kg.ha^{-1}$) treatments for both cultivars recorded a significant decrease in PPO activity at 4 months of growth followed by a significant increase. Polyphenol oxidases (PPOs) catalyse the oxidation of phenolic compounds into highly reactive quinones. Stress may account for most significant polyphenol oxidase activities in white cv plants after 6 months of growth and in red cv plants after 4 months of growth both treated with NPK_2 ($150kg.ha^{-1}$). Steffens (1994) suggested that *in vivo* polyphenol oxidase activity can be associated to senescing, wounding or damage to plant tissues in which cellular compartmentalization is lost. This is justified by the fact that classically polyphenol oxidases and their potential phenolic substrates are physically separated from one another in plants. Polyphenol oxidases are found in chloroplasts while phenolic compounds are found primarily in the vacuole and cell wall (Vaughn, 1988). Ioannis *et al.* (2019) had results which indicated that PPOs could accept flavonoids as their natural substrates and therefore might participate in the synthetic pathways of secondary metabolites.

At 2 months after planting both cultivars treated with NPK ($150kg.ha^{-1}$) recorded the most significant phenolic content values. NPK fertilizers might have increased soil salinity, leading to the formation of large amounts of oxygen free radicals ($O_2^{\bullet}$) in peroxisomes. Accumulation of reactive oxygen species (ROS) results in “oxidative stress”. ROS initiate free radical reactions that lead to oxidation of proteins, lipids and nucleic acids impairing their functions and causing cell death. This therefore justifies an increase in phenolic contents which form an antioxidant system developed by plants to counter “oxidative stress” by capturing ROS (Gill and Tuteja 2010; Sharma *et al*., 2012). Baaziz (2006) also depicted that total phenolic content in the extracts of *Pereskia bleo* leaves significantly influenced antioxidant and antimicrobial activities.

Significant flavonoid content values were recorded 6 months after planting in white cv plants and 4 months after planting in red cv plants both treated with poultry manure ($30t.ha^{-1}$). Flavonoids participate in plant protection against biotic (herbivores, microorganisms) and

abiotic stresses (UV radiation, heat), and due to their antioxidative properties, they also maintain a redox state in cells. The antioxidative activity of flavonoids is connected with the structure of the molecule: the presence of conjugated double bonds and the occurrence of functional groups in the rings (Seyoum *et al.*,2006; Ireneusz *et al.*, 2018; Wang, 2019). Flavonoids reduce the production of and quench reactive oxygen species (ROS) through: suppression of singlet oxygen; inhibition of enzymes that generate ROS (cyclooxygenase, lipoxygenase, monooxygenase, xanthine oxidase); chelating ions of transition metals, which may catalyze ROS production; quenching cascades of free-radical reactions in lipid peroxidation; "re-cycling" of other antioxidants (Rice-Evans *et al.*, 1996; Cotelle *et al.*, 1996 *et al.*, 1996; Arora *et al.*, 2000).

Recent advances in the pre-treatment procedures, separation techniques and spectrometry methods are used for qualitative and quantitative analysis of phenolic compounds. The online coupling of liquid chromatography with mass spectrometry (LC–MS) has become a useful tool in the metabolic profiling of plant samples (Bonta, 2017). One of the specific objectives of this work was to determine the effects of poultry manure and NPK fertilizers on the polyphenol profiles of white and red cv X. *sagittifolium* leaves during growth, using HPLC-DAD-MS. Eight flavonoid compounds with different retention times were identified on 4 peaks with varying intensities for all treatments. This implied that neither poultry manure nor NPK fertilizer influenced the synthesis of new flavonoid metabolites in *X. sagittifolium* leaves which were not found in control plants but stimulated the intensity of existing flavonoids. Osuagwu (2012) investigated the influence of inorganic fertilizer application on the flavonoid, phenol and steroid content in the leaves of *Ocimum gratissimum* and *Gongronema latifolium* and revealed that inorganic fertilizer treatments did not affect the presence of flavonoids, phenols and steroids in the leaves of these plants since these phytochemicals were present in both treated and untreated plants but affected their concentrations. The results show 8 flavonoid coumpounds identified amongst which 2 new specific flavone C- glycosides (isomers of apeginin) in *X. sagittifolium* and 6 known flavone C-glycosides (6,8-Di-C-glucopyranosylapigenin, Isovitexin 6"-O-glucopyranoside, apigenin 6-C-glucoside 8-C-arabinoside, vitexin, isovitexin and 2"-O-Malonylvitexin). These 2 new flavone C-glycosides (isomers of apigenin) could play important roles in the growth and development of plants. These new flavone C-glycosides can also be isolated and used in the pharmacological industry to produce drugs against cancer, diabetes and inflammations. The 6 known flavone C-glycosides were also identified by Picerno *et al.* (2003) in *Xanthosoma*

violaceum plants. They determined the polyphenol profile in *Xanthosoma violaceum* leaves and isolated a new flavone C-glycoside, apigenin 6-C-β-D-glucopyranosyl-8-C-β-D-apiofuranoside, as well as known flavone C-glycosides, including vitexin, isovitexin, isovitexin 4'-O-Orhamnopyranoside, apigenin 6-C-[β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside], and apigenin 6,8-di-C-β-D-glucopyranosid. Nevertheless, Ntengna *et al.* (2019) also identified apigenin-pentosyl-hexoside, apigenin-hexoside and apigeninrutinoside isomer in white cv *Xanthosoma sagittifolium* leaves while studying the effects of arbuscular mycorrhiza fungi on stimulation of nutrient content and induction of biochemical defense response in *Xanthosoma sagittifolium* plants against root rot disease caused by *Pythium myriotylum.* C- glycosyl structures are generally more resistant to hydrolysis, therefore fairly stable. These results suggest that C- glycosides of apigenin can be used as chemotaxonomic markers for the genus *Xanthosoma* (Gwan *et al.,* 2019). Further more apigenin appears to have the potential to be developed either as a dietary supplement or as an adjuvant chemotherapeutic agent for cancer therapy (Yan *et al.,* 2017). Based on the *in vitro* and *in vivo* evidence, apigenin, a natural bioactive flavone-type molecule, could play a key role in the prevention and treatment of emerging global health issues, highlighting once again the significant use of food components and/or plant compounds (Bahare *et al*., 2019).

Results from spectra analysis at 2 months of growth depicted that poultry manure treatments (30 $t.ha^{-1}$) and NPK fertilizer treatments (150 $kg.ha^{-1}$) recorded significant intensities for the first 3 peaks in white cv *X. sagittifolium* leaves while NPK fertilizer treatments ($150kg.ha^{-1}$) showed the most significant intensity values of the first 3 peaks in the red cv *X. sagittifolium* leaves as compared to the control treatments. At 6 months of growth NPK fertilizer treatments (150 $kg.ha^{-1}$) recorded significantly very reduced intensity values for the first and second peaks for both white cv and red cv *X. sagittifolium* leaves. These results agree with those obtained by Nithiya *et al*. (2015) who compared the effect of organic (vegetable waste, cattle dung) and inorganic fertilizer (NPK) on phytochemicals in *Solanum nigrum* and concluded that organic fertilizer treated plants have higher antioxidant activity than the inorganic fertilizer treated plants. Concordantly, Ibrahim *et al*. (2013) also studied the impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kacip Fatimah (*Labisia pumila* Benth) and illustrated that the use of chicken dung enhanced the production of total phenolics, flavonoids, ascorbic acid, saponin and gluthathione content in *L. pumila,* compared to the use of inorganic fertilizer. In addition, Elhanafi *et al.* (2019) evaluated the impact of excessive nitrogen fertilization on the

biochemical quality, phenolic compounds, and antioxidant power of *Sesamum indicum* L seeds and concluded that total phenolic, flavonoids content, and antioxidant activity showed a significant decrease. Various researchers have proved that flavonoids scale down diabetes mellitus either by avoiding glucose absorption or by improving glucose tolerance. *In vitro* experiments have stated that isoflavones of soyabean extract (daidzein and genistein) impede absorption of glucose in the small intestinal brush-border-membrane (BBM) vesicles of rabbits. Several flavonoids including apigenin ameliorate diabetes mellitus by inhibition of Na^+ dependent glucose transporter-1 (SGLT-1) (Rana and Gulliya, 2019).

Pearson correlation tests showed high positive correlation intensities between growth and yield parameters for both white and red cv *X. sagittifolium* plants treated with poultry manure ($30t.ha^{-1}$). The correlations between growth and yield parameters were generally non significant suggesting that many other parameters are involved in these physiological processes. In white cv *X. sagittifolium* plants treated with poultry manure ($30t.ha^{-1}$) between the different yield parameters were high, positive and very significant, indicating the direct involvement of these parameters in tuber yields. The correlations between the number of leaves and tuber number per plant was positive and significant for both cultivars. This is explained by the fact that leaves produce sugars during photosynthesis which are transported and stored in the tubers. Amarullah *et al.* (2016) also studied the correlation between growth and yield parameters of two Cassava (*Manihot esculenta* Crantz) varieties. Based on the correlation results, the growth parameters highly affected the final outcome or yield of cassava tubers at different growth phases. Principal component analysis (PCA) results, confirmed high positive correlations between soil exchangeable cations (Mg^{++} and Na^+) and average leaf number for both cultivars, thereby indicating their role in the biosynthesis of leaf tissues. Magnesium is a central component in the structure of chlorophyll which actively takes part in the process of photosynthesis. PCA results also illustrated that soil exchangeable K^+ were highly correlated to yield parameters (average tuber girth, length and weight). Plant roots absorb potassium ions from the soil and transport them in the xylem via the transpiration stream to the shoots. There, in source tissues where sufficient chemical energy (ATP) is available, K^+ is loaded into the phloem and then transported with the phloem stream to other parts of the plant like the roots. This further highlights the role of K^+ in the transport of sugars from leaves to storage organs. In addition, Ingo *et al*. (2017) showed that cycling of K^+ has been uncovered to be part of a sophisticated mechanism that enables the shoot to communicate its nutrient demand to the root, contributes to the K^+ nutrition of transport

phloem tissues and transports energy stored in the K^+ gradient between phloem, cytosol and the apoplast. This potassium battery can be tapped by opening AKT2-like potassium channels and then enables the ATP-independent energization of other transport processes, such as the reloading of sucrose.

IV.2 CONCLUSION

The objective of this pioneering field trial of *Xanthosoma sagittifolium* (white and red cultivars) minitubers as seeds was to evaluate their performance in response to different treatments of poultry manure and NPK fertilizers. Our results show that for all treatments, the white cultivar *X. sagittifolium* minitubers depicted better growth and yield parameters than the red cultivar minitubers. Poultry manure ($30t.ha^{-1}$) stimulated the most significant responses in terms of better growth and yield among all treatments in both cultivars, suggesting that poultry manure increases cation exchange capacities which offer higher nutrient contents and a more favourable pH to the soil than other treatments.

The results obtained from the macro mineral analysis of harvested tubers show that red cv Xanthosoma *sagittifolium* tubers recorded a higher content in calcium, magnesium, sodium, phosphorus and nitrogen than in white cv *Xanthosoma sagittifolium* tubers for all treatments. This suggests that red cv *Xanthosoma sagittifolium* tubers can serve as an essential dietary source for these minerals in addition to other traditional sources like cereals.

Both poultry manure ($30t.ha^{-1}$) and NPK ($150kg.ha^{-1}$) treatments in both cultivars significantly influenced total soluble protein content, peroxidase activity, glucanase activity, polyphenoloxidase activity, phenolic contents and flavonoid content in the leaves during growth. These secondary metabolites play key roles in controlling abiotic and biotic stress thereby enhancing growth and development in plants. This work serves as an initial step in the improvement of cocoyam (*Xanthosoma sagittifolium*) production in Cameroon using cocoyam minitubers as seeds. The present study clearly identified flavonoid profiles in the white and red cultivars of *Xanthosoma sagittifolium* leaves under different treatments of poultry manure and NPK fertilizers. All the compounds identified were flavone C-glycosides of apiginin, which could serve as chemotaxonomic markers of *Xanthosoma.* It was also observed that poultry manure treatments ($30t.ha^{-1}$) enhanced the production of these polyphenolic compounds after growth in the white and red cv plants. Recent literature has demonstrated that some of the identified compounds like vitexin (8-C-Glucosylapigenin) and isovitexin (6-C-Glucosylapigenin) have promising pharmacological effects against type II diabetes, gastric ulcers, cancer and other illnesses. Therefore, *Xanthosoma sagittifolium* leaves could be a useful source for the isolation and purification of these compounds, for use in the pharmacological industry.

IV.3 PERSPECTIVES

In order to better understand the effects of poultry manure and NPK fertilizers on field performance of *Xanthosoma sagittifolium* minitubers future research should focus on:

- HPLC- MS/MS analysis to isolate, purify and identify other secondary metabolites in the leaves during growth and cormels after harvest;
- Micro mineral analysis in harvested cormels to illucidate their dietary potential;
- Partnership with research institutions like IRAD in order to carryout multiple field tests of *Xanthosoma sagittifolium* minitubers in different geographical locations to determine performance as seeds;
- Proteomic analysis of *Xanthosoma sagittifolium* minitubers during growth and yield.

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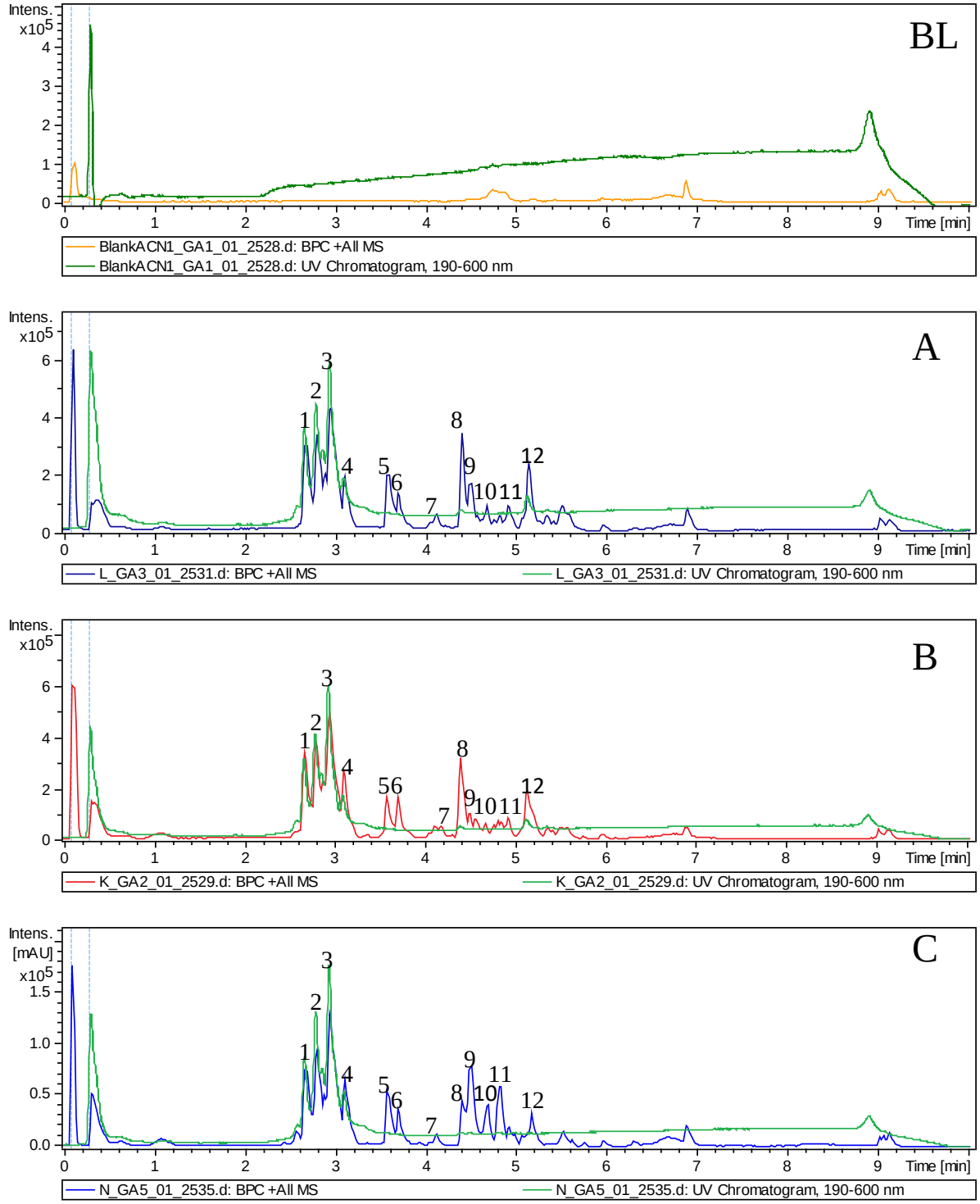
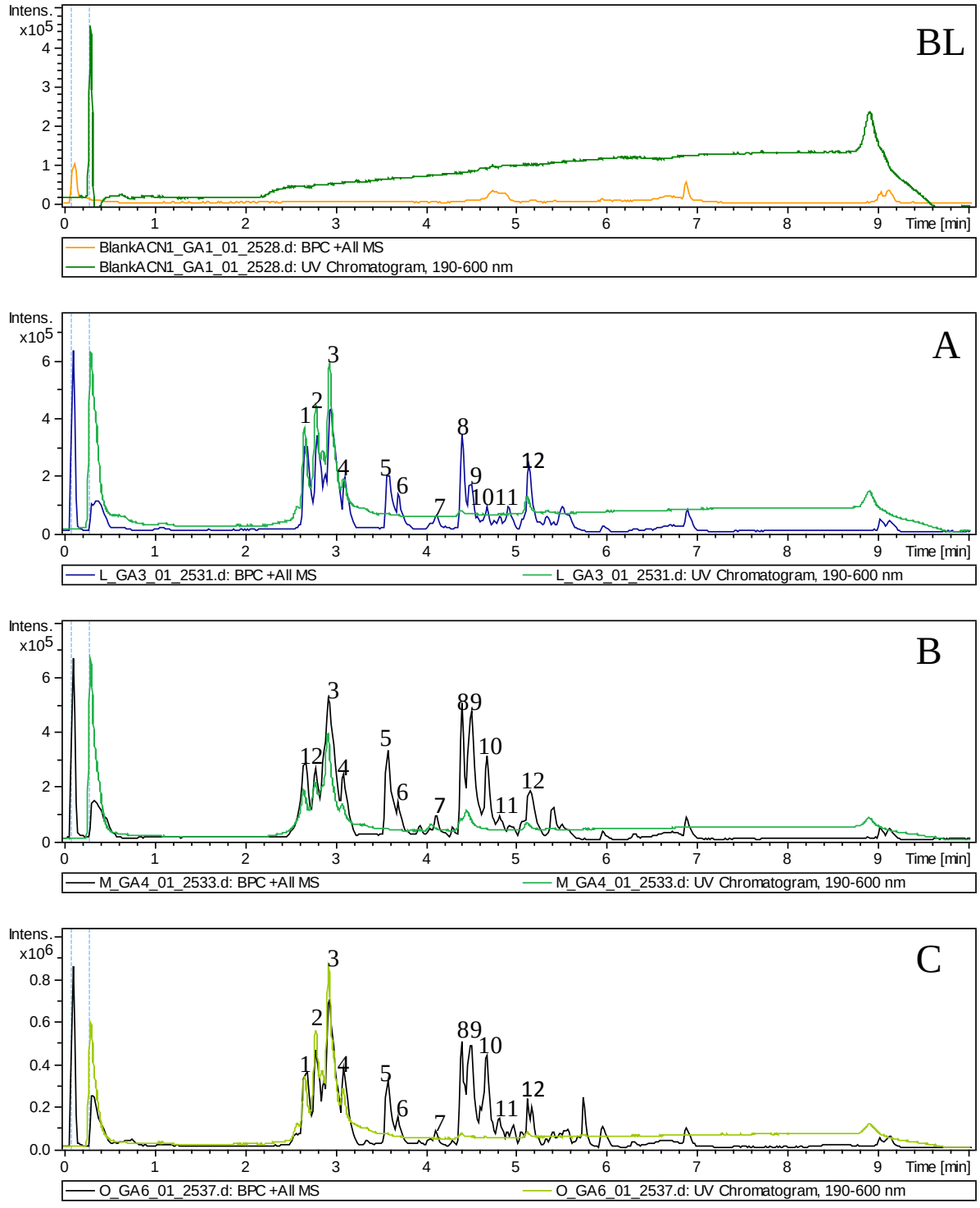
APPENDICES

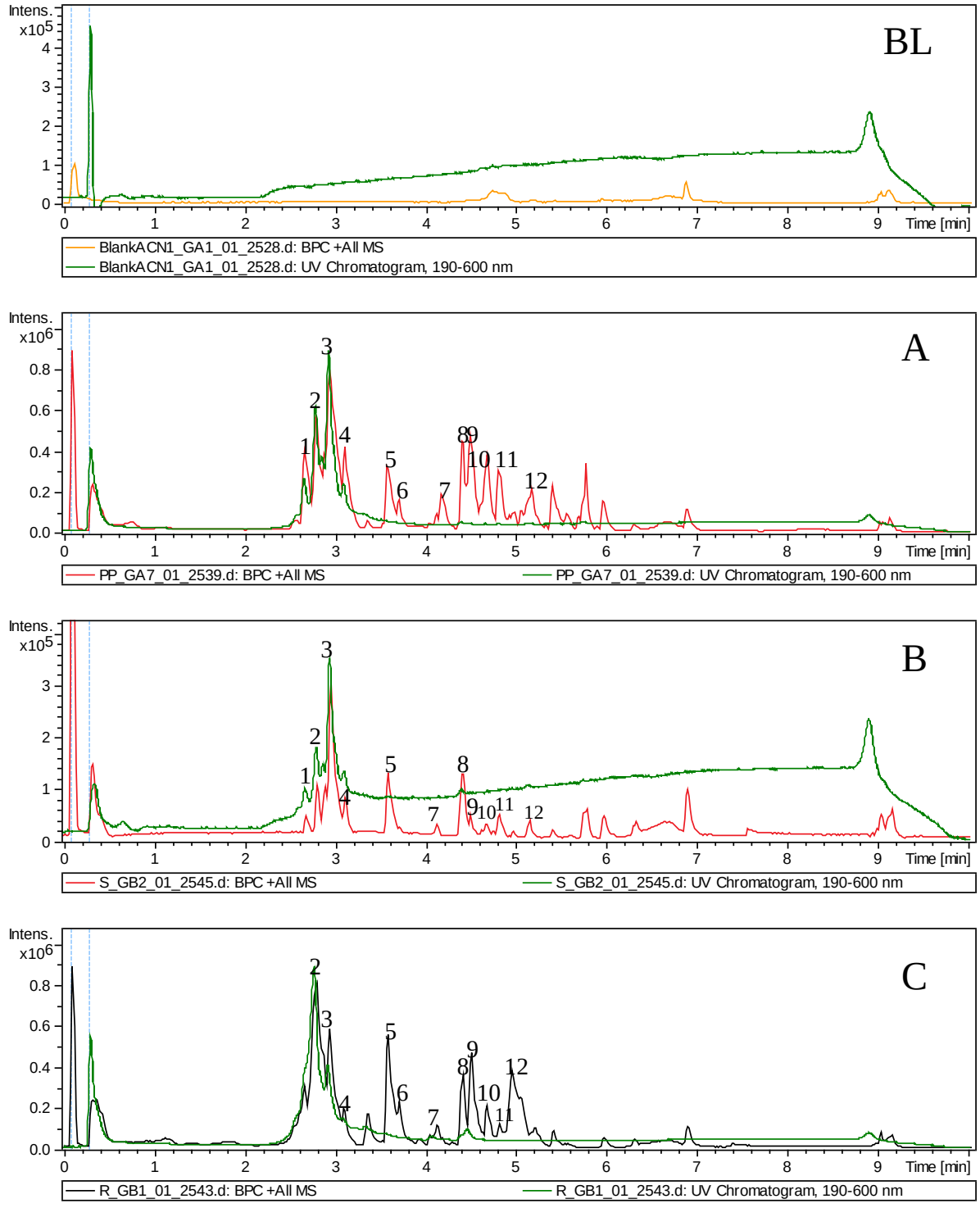
APPENDICES



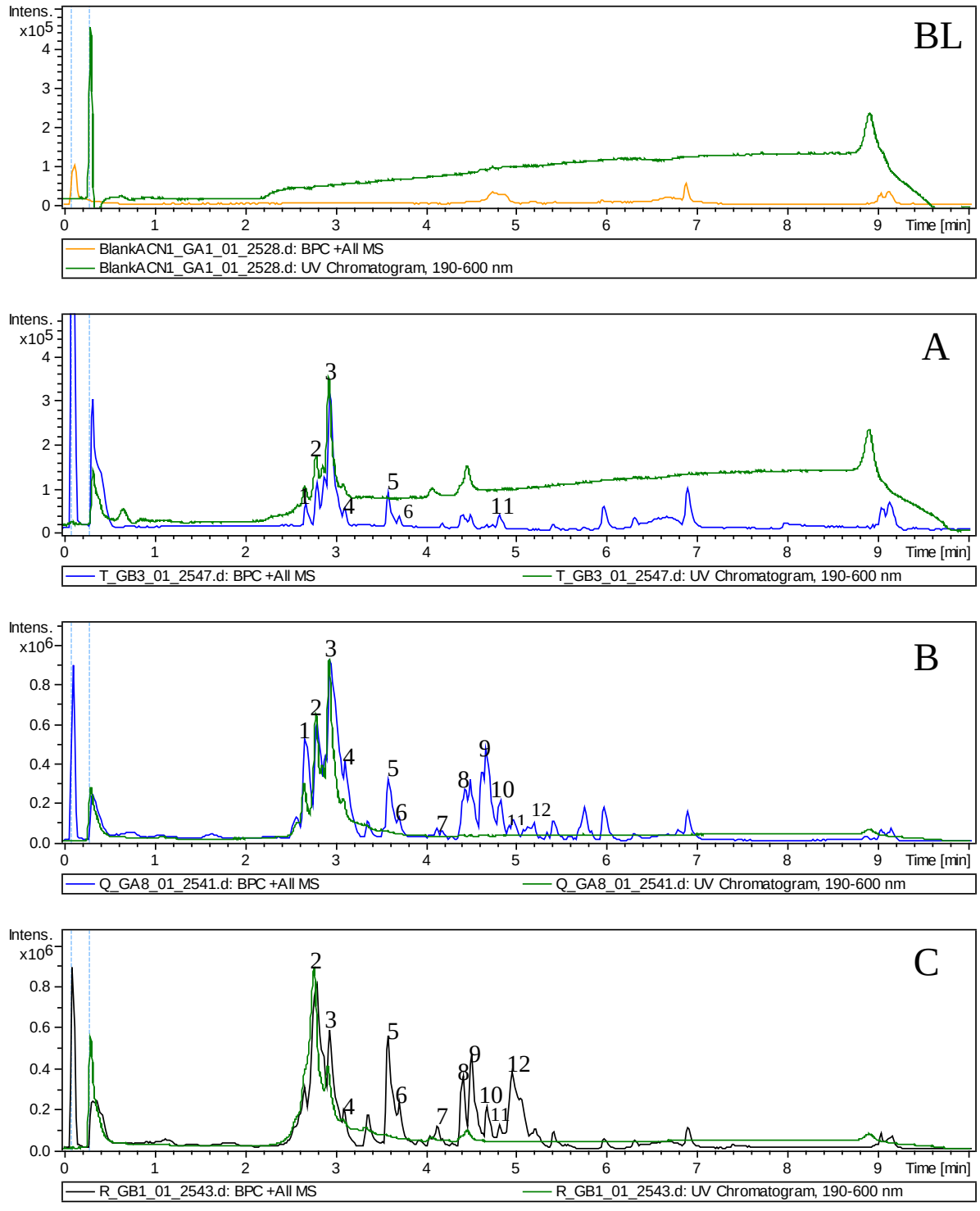
Appendix 1: Profiles of secondary metabolites from white cv leaves two months after planting. Blank (BL), Control (A), poultry manure (30t.ha^{-1}) (B) and NPK fertilizer (150kg.ha^{-1}) (C). and metabolite peaks (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12)



Appendix 2: Secondary metabolite profiles from red cv leaves 2 months after planting. Blank (BL), Control (A), poultry manure (30 $t.ha^{-1}$) (B) and NPK fertilizer (150 $kg.ha^{-1}$) (C) and metabolite peaks (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12)



Appendix 3: Secondary metabolite profiles from white cv leaves 6 months after planting. Blank (BL), Control (A), poultry manure (30 $t.ha^{-1}$) (B) and NPK fertilizer (150 $kg.ha^{-1}$) (C). Blank (B) and Metabolite peaks (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12)



Appendix 4**:** Secondary metabolite profiles from red cv leaves 6 months after planting. Blank (BL) Control (A), poultry manure (30 $t.ha^{-1}$) (B) and NPK fertilizer (150 $kg.ha^{-1}$) (C) and metabolite peaks (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12)

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PUBLICATIONS FROM THE THESIS

Gwan M.E., Djeuani A.C., Boudjeko T and Omokolo N.D. 2019. Different flavonoid profiles in *Xanthosoma sagittifolium* L. Schott leaves (white and red cv) during growth under the influence of poultry manure and NPK fertilizers. *International journal of science and research methodology* Human 13(3) 101-117.

Gwan M.E., Djeuani A.C., Tene Tayo P.M., Boudjeko T and Omokolo N.D. 2019. Field performance of *Xanthosoma sagittifolium* L. Schott minitubers grown under the influence of poultry manure and NPK fertilizers: Changes in content of some secondary Metabolites. *Journal of Biology, Agriculture and Healthcare* 9(20): 30-42.

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Different Flavonoid Profiles in *Xanthosoma sagittifolium* L. Schott Leaves (White and Red CV) During Growth under the Influence of Poultry Manure and NPK Fertilizers

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Gwan Mofor Elvis[*1,2], Djeuani Astride Carole[1,3], Boudjeko Thaddée[2,4], Omokolo Ndoumou Denis[1]

1. Department of Biological Sciences, Laboratory of plant physiology, Higher Teacher Training College (HTTC), University of Yaoundé I, Yaoundé-Cameroon.

2. Department of Biochemistry, Faculty of Science, University of Yaoundé I, Yaoundé-Cameroon.

3. Department of Plant Biology, Faculty of Science, University of Yaoundé I, Yaoundé-Cameroon.

4. Laboratory of Phytoprotection and Plant Valorization, Biotechnology Center, University of Yaoundé I, Yaoundé-Cameroon.

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ABSTRACT

Extracts from white and red cultivar *Xanthosoma sagittifolium* leaves treated with poultry manure (0t ha^{-1} and 30t ha^{-1}) and NPK 20:10:10 fertilizers (0kg ha^{-1} and 150kg ha^{-1}) were subjected to flavonoid profile determination using HPLC-DAD-MS. Analysis of the spectra obtained, identified eight flavonoid compounds in all treatments. The results illustrated two newly identified flavone C- glycosides (2 isomers of apeginin) and six known flavone C- glycosides (6,8-Di-C-glucopyranosylapigenin, Isovitexin 6"-O-glucopyranoside, Apigenin 6-C-glucoside 8-C-arabinoside, vitexin, isovitexin and. 2"-O-Malonylvitexin). The intensity of the first 3 peaks after 6 months of growth showed that poultry manure treatments (30t ha^{-1}) enhanced flavone production in the white and red cultivar *Xanthosoma sagittifolium* plants as compared to the NPK fertilizer and control treatments. These results show that glycosides of apeginin can be used as chemotaxonomic markers of *Xanthosoma.*

INTRODUCTION

Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plant fruits, leaves, grains, bark, roots, stems and flowers [1-2] and could also serve as chemotaxonomic marker compounds. Over 9000 flavonoids have been reported [3]. Flavonoids are frequently found as glycosylated or esterified forms, consisting of C6-C3-C6 rings, namely rings A and B linked by three carbon-ring C [2]. According to substitution pattern variations, flavonoids can thus be classified into different subclasses, providing an extremely diverse range of derivatives [4]. Due to their physical and biochemical properties, they are capable of participating in plants' interactions with other organisms (microorganisms or animals [2] and other plants [5]) and their reactions to environmental stresses [6]. Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV-filter, function as signal molecules, allelopathic compounds [7] phytoalexins, detoxifying agents [8], antimicrobial defensive compounds [9]. The majority of their functions result from their strong antioxidative properties [10]. From the aforementioned functions, it could be suggested that flavonoids play significant roles in plant growth. Flavonoids are also believed to have various bioactive effects including anti-viral, anti-inflammatory, cardioprotective, anti-diabetic, anti-cancer, anti-aging, in humans *etc* [4]. Some flavones like vitexin and isovitexin are active components of many traditional Chinese medicines and were found in various medicinal plants. Vitexin (apigenin-8-C-glucoside) has recently received increased attention due to its wide range of pharmacological effects, including but not limited to anti-oxidant, anti-cancer, anti-inflammatory, anti-hyperalgesic, and neuroprotective effects. Isovitexin (apigenin-6-C-glucoside), an isomer of vitexin, generally purified together with vitexin, also exhibits diverse biological activities [11]. [12] Conducted oral glucose tolerance and antinociceptive activity tests with methanol extracts (containing apigenin and its sugar derivatives) from *Xanthosoma violaceum* aerial parts and concluded that bioactive components in these extracts like apigenin, vitexin and isovitexin could be used to lower blood sugar levels in diabetic patients and alleviate pain. [13] Also identified derivatives of apigenin in cocoyam (*Xanthosoma sagittifolium*) leaves.

Cocoyam (*Xanthosoma sagittifolium* L. Schott, Araceae) is a herbaceous plant cultivated in tropical and subtropical regions for its edible tubers and leaves. *X. sagittifolium* leaves contain significant antioxidant compounds which have anti-diabetic properties [14] and can also be used to treat gastrointestinal illnesses [15]. The increasing decline in soil fertility

levels and lack of soil management practices has significantly reduced *X. sagittifolium* production. *X. sagittifolium* responds very well to input of fertilizer whether organic or inorganic as reported by several workers [16-18]. Different manures (fish, pigeon and cow) and synthetic fertilizers (nitrogen) have been shown to influence some biochemical activities like phenolic constituents of plants [19]. The quantity of phenolic and polyphenolic compounds present in a given species of plant material varies with a number of factors such as cultivar, environmental conditions, cultural practices postharvest storage and processing [20]. It is in this logic that this investigation was carried out to evaluate the effects of poultry manure and NPK fertilisers on the profiles of flavonoids in the leaves of white and red cultivar *X. sagittifolium* plants during growth while highlighting the possible flavonoids which can serve as chemotaxonomic marker compounds for cocoyam (*X. sagittifolium*).

MATERIALS AND METHODS

Site location

A field trial was conducted to study the performance of *Xanthosoma sagittifolium* mini tuber seeds as influenced by poultry manure and NPK fertilizer during the 2017 cropping season on an experimental farm at Ngog Bibega, Mbankomo Sub-division, (Outskirsts of Yaoundé) Centre region, Cameroon, located at latitude 3°49′52.54″N and longitude 11°27′15.79″E and 714 m above sea level. The area is characterized as a humid rainforest zone and the soil is clay loam. The total annual rainfalls for 2017 was 1902.8 mm while the total rainfalls during the period of experimentation (April to December) for 2017 was 1775.6 mm.

Materials

The planting material consisted of white and red cultivars of *X. sagittifolium* mini tuber seeds of mean weight 38g produced from acclimatised vitro plants under the shed in the plant physiology Laboratory of the Higher Teachers Training college (HTTC), University of Yaounde I. Yaounde, Cameroon [21-22]. The Poultry manure was obtained from Henri et Freres Poultry farm Yaoundé, Cameroon while the NPK fertilizer (20:10:10) was obtained from the fertilizer unit of the Centre Regional Delegation of Agriculture and Rural Development, Yaoundé, Cameroon.

Experimental design and treatments.

The experiment was a 4×2 factorial arrangements in a randomized complete block design and replicated three times. The site was ploughed, ridged and marked out into two main blocks, one for each cultivar. Each of these main blocks was further subdivided into 3 sub blocks which represent the three replicates. Each sub block was divided into three experimental plots, thus a total of nine plots were used for cultivar. Each gross plot measured 4m×3m (12 m^2) with a net plot of 2m×2m. The treatments comprised two rates each application of Poultry Manure (0, and 30 t ha-1) and NPK fertilizer (0, and 150 kg ha-1). A total of 9 treatment combinations and three replications were used. The Poultry manure was incorporated into the soils on the experimental plots in a single application based on the treatment combinations, at 2 weeks before planting to ease decomposition, while the NPK fertilizer was applied to the cocoyam stands according to treatment allocation at 4 weeks after planting (WAP) using the ring placement method. Each mini tuber was planted per hole at a depth of 15 cm and at a spacing of 0.5 m x 1.0m resulting to about twenty-five plants per plot and a total of 250 plants per cultivar. All plots were kept weed free by manual weeding. Five cocoyam plants were randomly selected from each of the net plots, tagged and the harvested leaves at 2 months and 6 months after planting were used for the determination of the flavonoid profiles.

Extraction of total phenolic compounds

The extraction of the content of total phenolic compounds were performed as described by [23] with modification. Total phenolic compounds were extracted twice using 80% methanol. One gram of fresh *X. sagittifolium* leaves was ground in 10 ml of 80% methanol at 4˚C. After 5 min of agitation, the ground material was centrifuged at 10,000 g for 5 min at 4˚C. The supernatant was collected, and the pellet was re-suspended in 5 ml of 80% methanol followed by agitation for 5 min. After the second centrifugation at 4˚C, the supernatant was collected and mixed with the previously collected supernatant to constitute the phenolic extract.

Qualitative determination of compound contents from *X. sagittifolium* leaves using HPLC-DAD-(HR) ESI-MS

Sample preparation

Aqueous preparation extracts were separately dissolved in HPLC grade methanol in a concentration of 5 mg/ml then filtrated through a syringe-filter-membrane. Aliquots of 5 µl were injected into the LC-DAD/MS Dionex Ultimate 3000 HPLC (Germany), used for performing the analyses.

HPLC-MS conditions

High resolution mass spectra were obtained with an OTOF Spectrometer (Bruker, Germany) equipped with a HRESI source and a UV-vis absorbance detector. The spectrometer was operated in positive mode (mass range: 100-1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation using Na Formate as calibrant. Mass spectra were simultaneously acquired using electrospray ionization in the positive ionization mode. The following parameters were used for experiments: spray voltage of 4.5 kV, capillary temperature of 200°C. Nitrogen was used as sheath gas (10 l/min). The spectrometer was attached to an Ultimate 3000 (Thermo Fisher, USA) HPLC system consisting of LC-pump, UV traces were measured at 215, 218, 254, 280 and 330 nm and UV spectra-Diode Array Detector-(DAD) were recorded between 190 and 600 nm, autosampler (injection volume 5 µl) and column oven (35,0 °C). The separations were performed using a Synergi MAX-RP 100A (50x2 mm, 2.5µ particle size) with a H_2O (+0.1% HCOOH) (A)/acetonitrile (+0.1% HCOOH) (B) gradient (flow rate 500 µL/min). Samples were analyzed using a gradient program as follows: 95% A isocratic for 1.5min, linear gradient to 100 % B over 6 min, after 100% B isocratic for 2min, the system returned to its initial condition (90 % A) within 1 min and was equilibrated for 1 min.

Identification of peaks

Identification of all constituents was performed by HPLC-DAD-MS/MS analysis and by comparing the UV, MS spectra and MS/MS fragmentation of the peaks in the samples with those of data reported in the literature of Scifinder database.

RESULTS

Flavonoids were determined in the fluorescent region of the spectra. Four peaks with varying intensities were identified in the spectra of all treatments. In the first peak, two metabolites with a molecular formula of $C_{27}H_{30}O_{15}$ and an average molecular weight of 595.16g/mol were identified. 6,8-Di-C-glucopyranosylapigenin (Fig. 2A) was identified with a retention time of 2.63 min with 3 absorption wavelengths peaks (210nm, 270nm and 333nm) while Isovitexin 6"-O-glucopyranoside (Fig. 2B) had a retention time of 2.72 min with 3 absorption wavelength peaks (214nm, 270nm and 335nm) (Table 1).

In the second peak, three metabolites with a molecular formula of $C_{26}H_{28}O_{14}$ and an average molecular weight of 565.15 $g.mol^{-1}$were recorded. The first metabolite on this peak Apigenin 6-C-glucoside 8-C-arabinoside (Schaftoside) (Fig. 3) had a retention time of 2.82 min with 3 absorption wavelength peaks (214nm, 270nm and 235nm). The second metabolite Apigenin-6-C-pentoside-8-C- hexoside (Isomer 1) (Fig. 3) with a retention time of 2.84 min and 3 absorption wavelength peaks (214nm, 270nm and 339nm) while the third metabolite Apigenin-6-C-pentoside-8-C- hexoside (Isomer 2) (Fig.3) recorded a retention time of 2.90 min. with 3 absorption wavelength peaks (214nm, 270nm and 335nm) (Table 1). The third peak recorded two metabolites having a molecular formula of $C_{21}H_{20}O_{10}$ and average molecular weight of 433.11 $g.mol^{-1}$. 8-C-Glucosylapiginin (vitexin) (Fig. 4A) had a retention time of 2.97min with 3 absorption wavelength peaks (210nm, 270nm and 339nm) while 6-C-Glucosylapiginin (isovitexin) (Fig. 4B) recorded a retention time of 3.06 min with 3 absorption wavelength peaks (210nm, 270nm and 335nm) (Table 1). One metabolite was identified at the fourth peak with a molecular formula of $C_{24}H_{22}O_{13}$ and average molecular weight 519.11$g.mol^{-1}$. 2"-O-Malonylvitexin (Fig. 5) recorded a retention time of 3.13 min with 3 absorption wavelength peaks (210nm, 270nm and 335nm) (Table 1).

Flavonoid profile results show that there was a general increase in peak intensities between 2 and 6 months of growth for all treatments in both, white and red cv of *X. sagittifolium* leaves (Table 2). Analysis of the spectra obtained from the white cv of *X. sagittifolium* leaves after 2 months of planting revealed that the intensities of the first 3 peaks between the retention times of 2.63 min and 3.06 min were more significant in poultry manure treatments (30 $t.ha^{-1}$) and NPK fertilizer treatments (150 $kg.ha^{-1)}$ as compared to the control treatments (0 $kg.ha^{-1}$) (Fig. 6 and Table 2). Anyway, the spectra from the red cv of *X. sagittifolium* leaves after 2 months of planting also showed that the intensities of the first 3 peaks between the retention

times of 2.63 min and 3.06 min were most significant in the NPK fertilizer treatments (150kg.ha$^{-1)}$ than in the control treatments (0 kg.ha^{-1}) and the poultry manure treatments (30 t.ha^{-1}) (Fig. 7 and Table 2).

Six months after planting the spectra obtained from both white and red cv of *X. sagittifolium* leaves illustrated that the first and second peaks with retention times between 2.63 min and 2.9 min were significantly reduced in intensity with the NPK fertilizer treatments (150 kg.ha^{-1}) as compared the control treatments (0 kg.ha^{-1}) and the poultry manure treatments (30 t.ha^{-1}). The third peak recorded the most significant intensity values with poultry manure treatments (30t ha^{-1}) when compared to the control treatments (0 kg.ha^{-1}) and NPK fertilizer treatments (150 kg.ha^{-1}) (Fig.8, Fig. 9 and Table 2).

Table No. 1: Spectra analysis

N°	Rt (min)	$[M+H]^+$			UV, λ_{max} (nm)	Formular	Metabolites
		Exp.	Calcd.	Δ(*ppm*)			
01	2.63	595.1690	595.1557	2.4	210 270 335	$C_{27}H_{30}O_{15}$	6,8-Di-C-glucopyranosylapigenin
02	2.72				214 270 335		Isovitexin 6"-O- glucopyranoside
03	2.82	565.1585	565.1552	2.1	214 270 335	$C_{26}H_{28}O_{14}$	Apigenin 6-C-glucoside 8-C-arabinoside
04	2.84				214 270 339		**Apigenin-6-C-pentoside-8-C- hexoside (Isomer 1)**
05	2.90				214 270 335		**Apigenin-6-C-pentoside-8-C- hexoside (Isomer 2)**
06	2.97	433.1152	433.1129	3.9	210 270 339	$C_{21}H_{20}O_{10}$	8-C-Glucosylapigenin (vitexin)
07	3.06				210 270 335		6-C-Glucosylapigenin (Isovitexin)
08	3.13	519.1169	519.1133	0.8	210 270 335	$C_{24}H_{22}O_{13}$	2"-O-Malonylvitexin

Table No. 2: Peak Flavonoid intensities in *X. sagittifolium* leaves

		Peak intensity (mAU) 2 months after planting			Peak intensity (mAU) 6 months after planting		
leaves	Peak (N^o)	Control	Poultry manure	NPK	Control	Poultry manure	NPK
X. sagittifolium (White cv)	**1**	0.8 x 10^5	1.1 x 10^5	1.0 x 10^5	1.0 x 10^5	1.1 x 10^5	0
	2	1.0 x 10^5	1.4 x 10^5	1.4 x 10^5	2.4 x 10^5	2.4 x 10^5	0
	3	1.3x 10^5	2.1 x 10^5	1.9 x 10^5	3.4 x 10^5	4.4 x 10^5	3.6 x 10^5
	4	0.4 x 10^5	0.55 x 10^5	0.5 x 10^5	1.0 x 10^5	1.5 x 10^5	1.6 x 10^5
X. sagittifolium (Red cv)	**1**	0.8 x 10^5	0.65 x 10^5	1.5 x 10^5	1.1 x 10^5	1.2 x 10^5	0
	2	1.05 x 10^5	0.7 x 10^5	2.5 x 10^5	2.3 x 10^5	2.4 x 10^5	0
	3	1.28x 10^5	1.45 x 10^5	3.8 x 10^5	3.4 x 10^5	4.4 x 10^5	3.68 x 10^5
	4	0.41 x 10^5	0.5 x 10^5	0.6 x 10^5	0.8 x 10^5	1.49 x 10^5	1.6 x 10^5

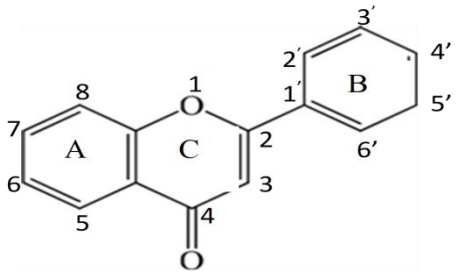

Fig No. 1: Basic structure of flavones (Rana and Gulliya, 2019)

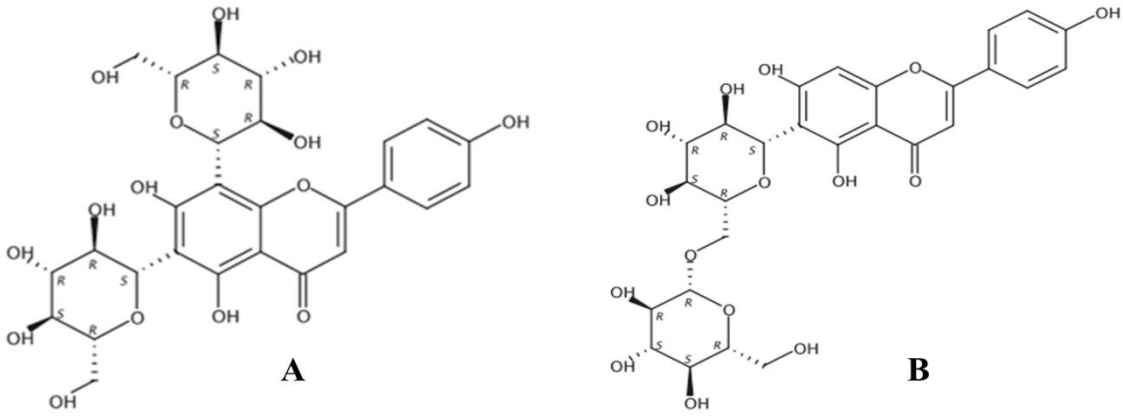

Fig No. 2: Flavone metabolite structures with same molecular formula ($C_{27}H_{30}O_{15}$) at 2.36 and 2.72 (min) retention times. 6,8-Di-C-glucopyranosylapigenin (A) and Isovitexin 6''-O-glucopyranoside (B).

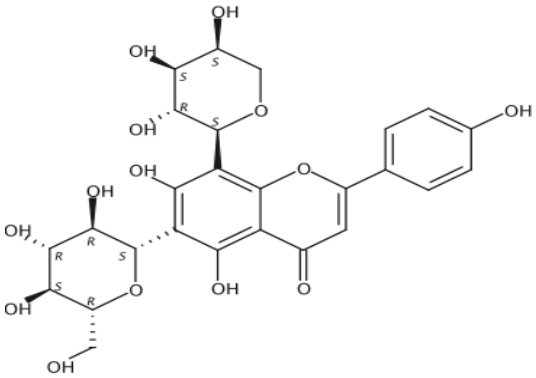

Fig No. 3: Isomer structures of three metabolite flavones with same molecular formular ($C_{26}H_{28}O_{14}$) at 2.82, 2.84 and 2.90 (min) retention times. Apigenin 6-C-glucoside 8-C-arabinoside or Apigenin-6-C-pentoside-8-C- hexoside (Isomer 1) or Apigenin-6-C-pentoside-8-C- hexoside (Isomer 2).

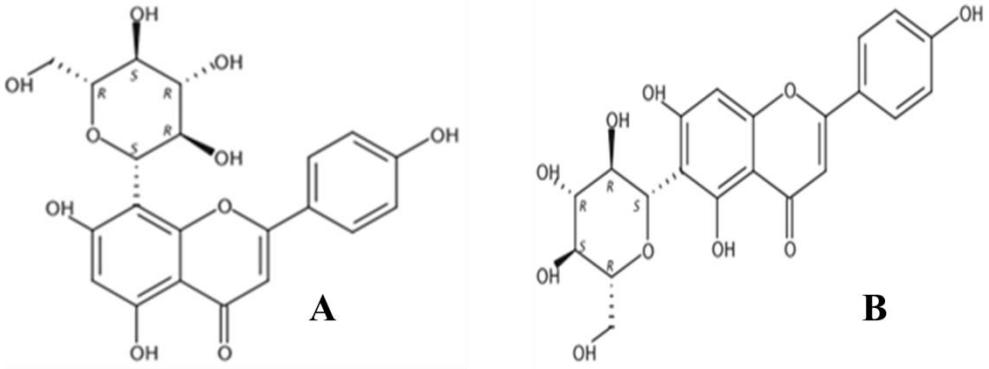

Fig. No. 4: Flavone metabolite structures with same molecular formular ($C_{21}H_{20}O_{10}$) at 2.97 and 3.06 (min) retention times. 8-C-Glucosylapigenin (vitexin) (A) and 6-C-Glucosylapigenin (Isovitexin) (B).

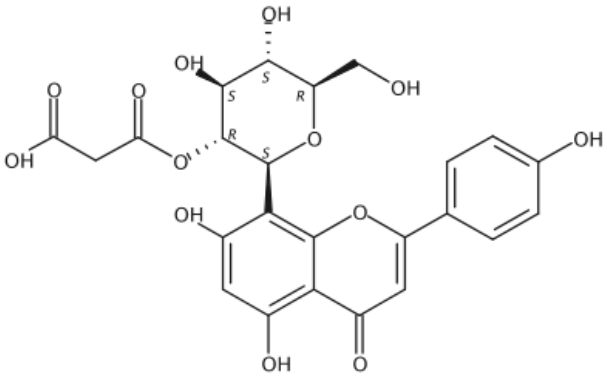

Fig. No. 5: Flavone metabolite structure of 2''-O-Malonylvitexin with $C_{24}H_{22}O_{13}$ molecular formular at 3.13 (min) retention time.

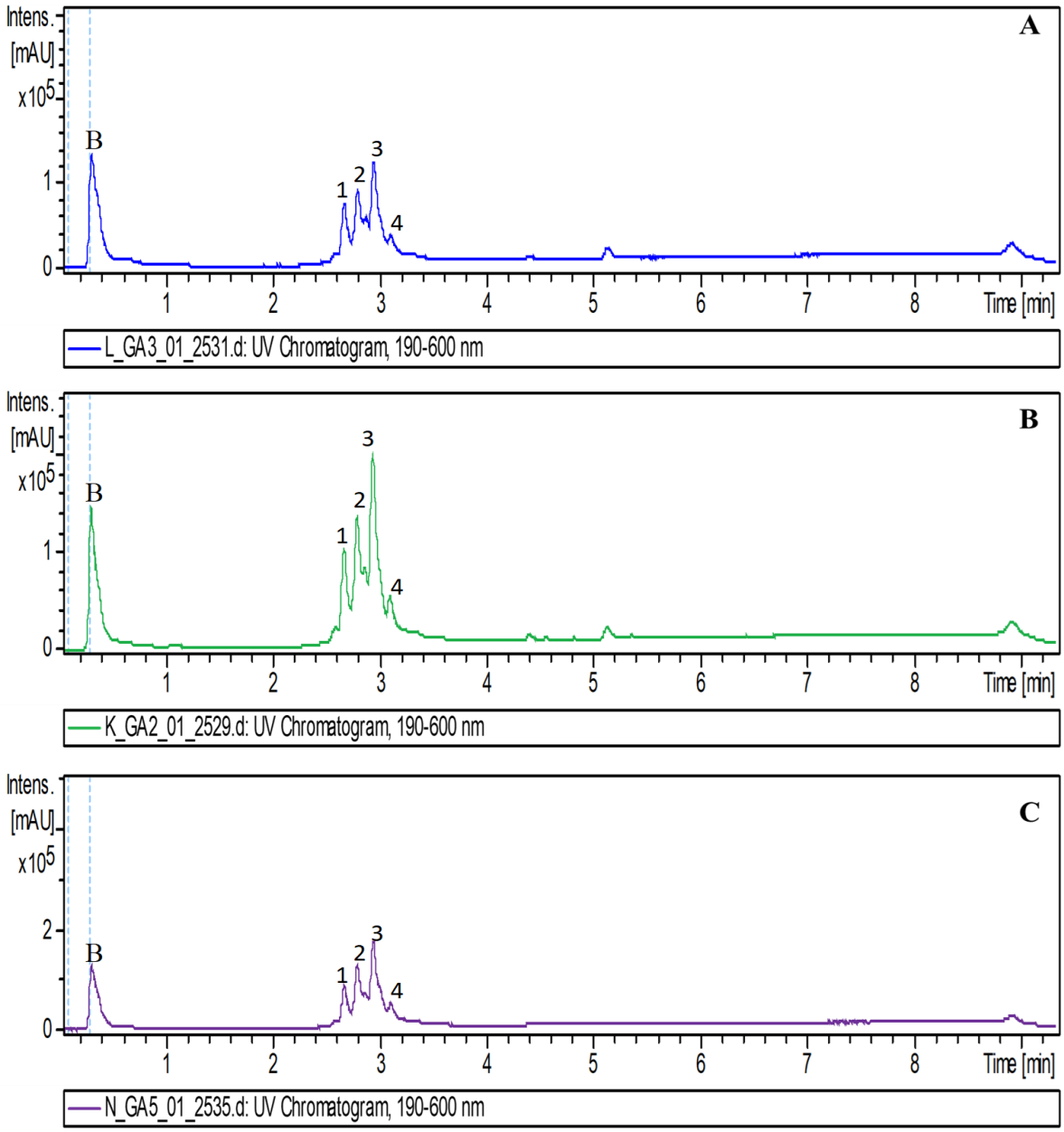

Fig. No. 6: Flavonoid profiles from white cv leaves two months after planting. Control (A), poultry manure (30t ha^{-1}) (B) and NPK fertilizer (150kg ha^{-1}) (C). Blank (B) and flavonoid peaks (1,2,3 and 4).

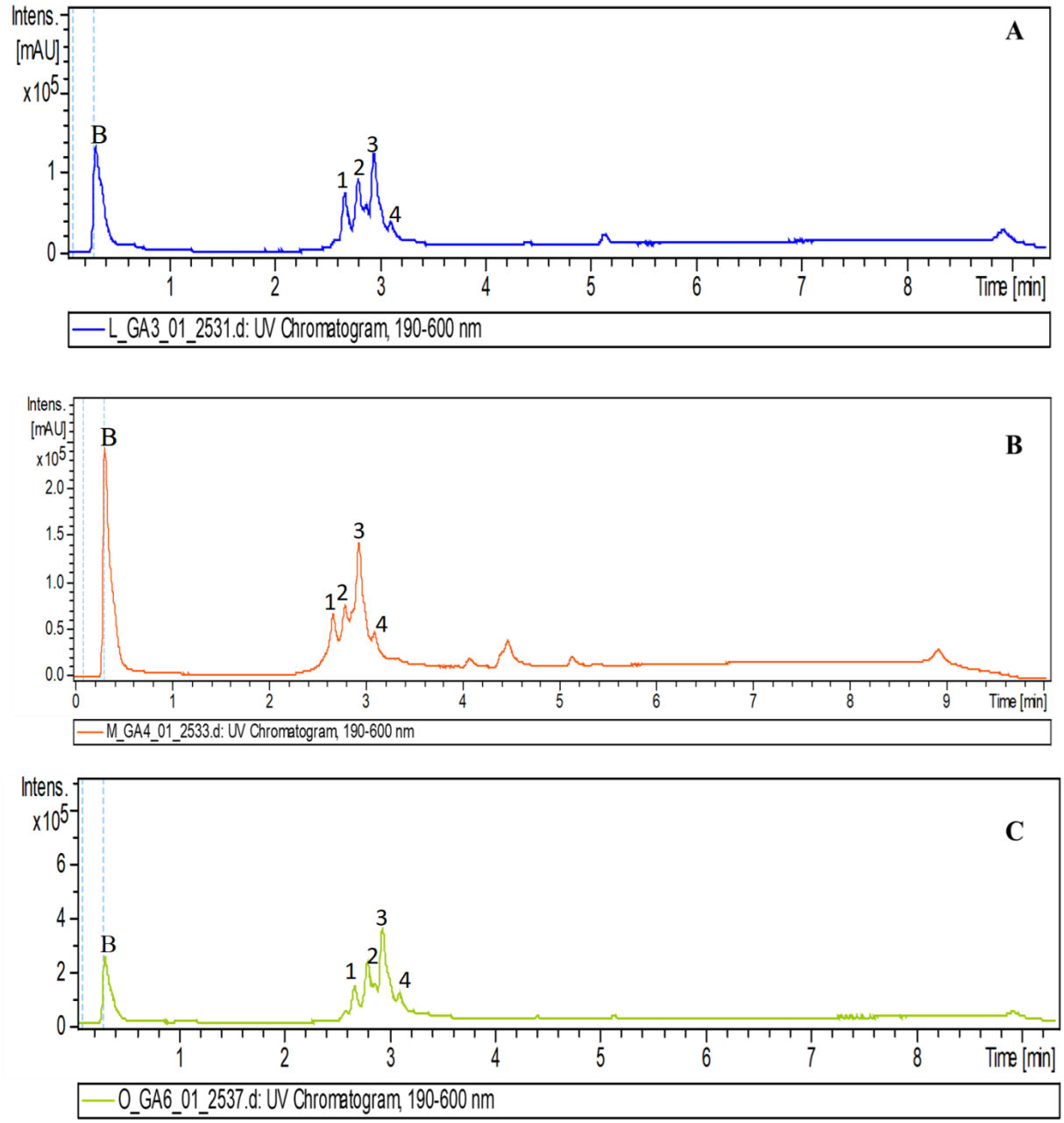

Fig. No. 7: Flavonoid profiles from red cv leaves 2 months after planting. Control (A), poultry manure (30t ha^{-1}) (B) and NPK fertilizer (150kg ha^{-1}) (C). Blank (B) and flavonoid peaks (1,2,3 and 4).

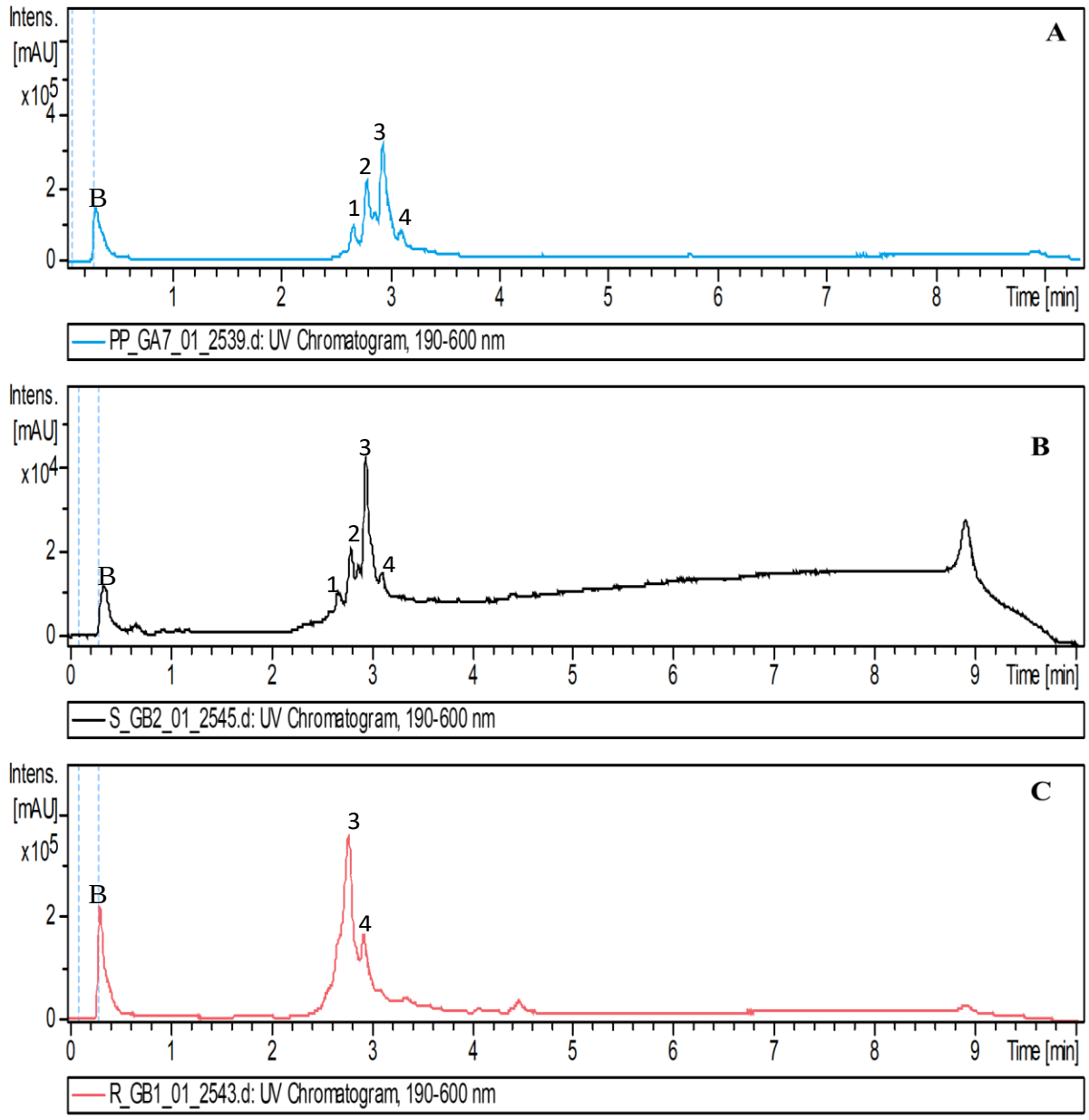

Fig. No. 8: Flavonoid profiles from white cv leaves 6 months after planting. Control (A), poultry manure (30t ha^{-1}) (B) and NPK fertilizer (150kg ha^{-1}) (C). Blank (B) and flavonoid peaks (1,2,3 and 4).

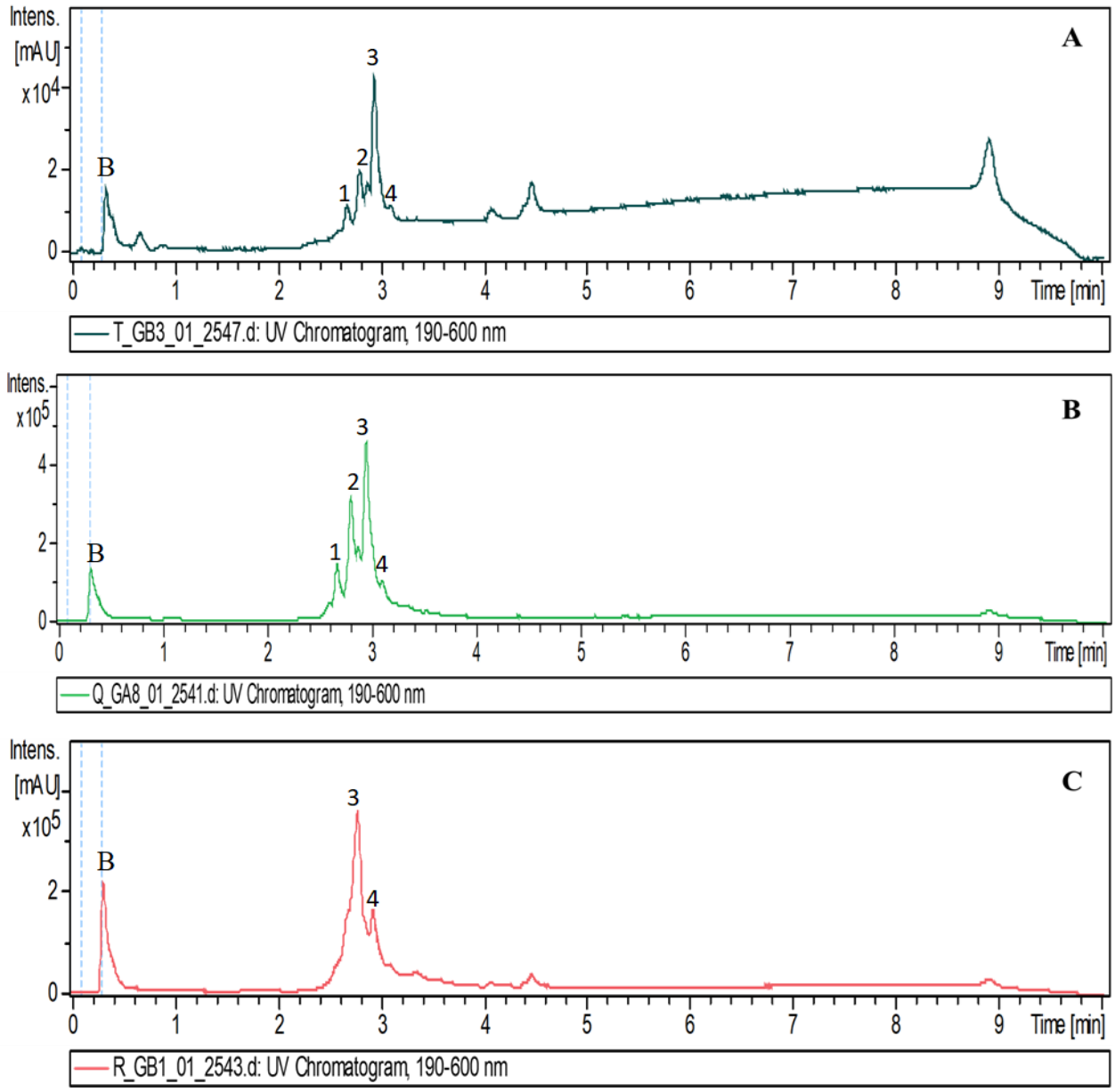

Fig. No. 9: Flavonoid profiles from red cv leaves 6 months after planting. Control (A), poultry manure (30t ha^{-1}) (B) and NPK fertilizer (150kg ha^{-1}) (C). Blank (B) and flavonoid peaks (1,2,3 and 4).

DISCUSSION

Recent advances in the pre-treatment procedures, separation techniques and spectrometry methods are used for qualitative and quantitative analysis of phenolic compounds. The online coupling of liquid chromatography with mass spectrometry (LC-MS) has become a useful tool in the metabolic profiling of plant samples [24]. The objective of this work was to

determine the effects of poultry manure and NPK fertilizers on the flavonoid profiles of white and red cv *X. sagittifolium* leaves during growth, using HPLC-DAD-MS. Eight flavonoid compounds with different retention times were identified on 4 peaks with varying intensities for all treatments. This implied that neither poultry manure nor NPK fertilizer influenced the synthesis of new flavonoid metabolites in *X. sagittifolium* leaves which were not found in control plants but stimulated the intensity of existing flavonoids. Similarly, [25] investigated the influence of inorganic fertilizer application on the flavonoid, phenol and steroid content in the leaves of *Ocimum gratissimum* and *Gongronema latifolium* and revealed that inorganic fertilizers treatments did not affect the presence of flavonoids, phenols and steroids in the leaves of these plants since these phytochemicals were present in both treated and untreated plants but affected their concentrations. The results show 8 flavonoid compounds identified amongst which 2 new specific flavone C- glycosides (isomers of apeginin) in *X. sagittifolium* and 6 known flavone C- glycosides (6,8-Di-C-glucopyranosylapigenin, Isovitexin 6"-O-glucopyranoside, apigenin 6-C-glucoside 8-C-arabinoside, vitexin, isovitexin and. 2"-O-Malonylvitexin. These 2 new flavone C- glycosides (isomers of apigenin) could play important roles in the growth and development of plants. These new flavone c- glycosides can also be isolated and used in the pharmacological industry to produce drugs against cancer, diabetes and inflammations. The 6 known flavone C- glycosides were also identified by [26]. They determined the polyphenol profile of *Xanthosoma violaceum* leaves and isolated a new flavone C-glycoside, apigenin 6-C-β-D-glucopyranosyl-8-C-β-D-apiofuranoside, as well as known flavone C-glycosides, including vitexin, isovitexin, isovitexin 4'-O-Orhamnopyranoside, apigenin 6-C-[β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside], and apigenin 6,8-di-C-β-D-glucopyranosid. Nevertheless, [13] also identified apigenin-pentosyl-hexoside, apigenin-hexoside and apigeninrutinoside isomer in white cv *Xanthosoma sagittifolium* leaves while studying the effects of arbuscular mycorrhiza fungi on stimulation of nutrient content and induction of biochemical defense response in *Xanthosoma sagittifolium* plants against root rot disease caused by *Pythium myriotylum.* These results suggest that glycosides of apigenin can be used as chemotaxonomic markers for the genus *Xanthosoma.*

Results from spectra analysis at 2 months of growth depicted that poultry manure treatments (30 $t.ha^{-1}$) and NPK fertilizer treatments (150 $kg.ha^{-1}$) recorded significant intensities for the first 3 peaks in white cv *X. sagittifolium* leaves while NPK fertilizer treatments (150 $kg.ha^{-1}$) showed the most significant intensity values of the first 3 peaks in the red cv *X. sagittifolium*

leaves as compared to the control treatments. At 6 months of growth NPK fertilizer treatments (150 $kg.ha^{-1}$) recorded significantly very reduced intensity values for the first and second peaks for both white cv and red cv *X. sagittifolium* leaves. These results agree with those obtained by [27] who compared the effect of organic (vegetable waste, cattle dung) and inorganic fertilizer (NPK) on phytochemicals in *Solanum nigrum* and concluded that organic fertilizer treated plants have higher antioxidant activity than the inorganic fertilizer treated plants. Concordantly, [28] also studied the impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kacip Fatimah (*Labisia pumila* Benth) and illustrated that the use chicken dung enhances the production of total phenolics, flavonoids, ascorbic acid, saponin and glutathione content in *L. pumila*, compared to the use of inorganic fertilizer. In addition, [29] evaluated the impact of excessive nitrogen fertilization on the biochemical quality, phenolic compounds, and antioxidant power of *Sesamum indicum* L seeds and concluded that total phenolic, flavonoid content, and antioxidant activity showed a significant decrease.

CONCLUSION

The present study clearly identified flavonoid profiles in the white and red cultivars of *Xanthosoma sagittifolium* leaves under different treatments of poultry manure and NPK fertilizers. All the compounds identified were flavone C-glycosides of apiginin, which could serve as chemotaxonomic markers of *Xanthosoma.* It was also observed that poultry manure treatments (30 $t.ha^{-1}$) enhanced the production of these polyphenolic compounds after growth in the white and red cv plants. Recent literature has demonstrated that some of the identified compounds like vitexin and isovitexin have promising pharmacological effects against type II diabetes, gastric ulcers, cancer and other illnesses. Therefore, *Xanthosoma sagittifolium* leaves could be a useful source for the isolation and purification of these compounds, for use in the pharmacological industry.

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CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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Field Performance of Xanthosoma sagittifolium L. Schott Minitubers Grown Under the Influence of Poultry Manure and NPK Fertilizers: Changes in Content of Some Secondary Metabolites

Gwan Mofor Elvis[1,2*] Djeuani Astride Carole[1,3] Tene Tayo Paul Martial[2,4] Boudjeko Thaddée[2,4] Omokolo Ndoumou Denis[1]

1.Department of Biological Sciences, Laboratory of plant physiology, Higher Teacher Training College (HTTC), University of Yaoundé I, Yaoundé-Cameroon

2.Department of Biochemistry, Faculty of Science, University of Yaoundé I, Yaoundé-Cameroon

3.Department of Plant Biology, Faculty of Science, University of Yaoundé I, Yaoundé-Cameroon

4.Laboratory of Phytoprotection and Plant Valorization, Biotechnology Center, University of Yaounde I, Yaoundé- Cameroon

Abstract

The response of white and red cultivar (cv) cocoyam *(Xanthosoma sagittifolium* (L.) Schott) minituber seeds to different rates of poultry manure (PM) and NPK (20:10:10) fertilizer was studied under field conditions in the 2017 cropping season on an experimental farm at Ngog Bibega, Mbankomo Sub-division, (outskirsts of Yaounde) Centre region, Cameroon. Treatment combinations comprising of three rates each of poultry manure (0, 20 and 30t ha^{-1}) and NPK fertilizer (0, 120 and 150 kg ha^{-1}) were factorized and fitted into a randomized complete block design with three replicates. Physico-chemical analysis of all treatment plots revealed clay loam textures and poultry manure treatment plots significantly ($P<0.05$) increased soil pH, organic content, total carbon and cation exchange capacity while NPK treatments significantly ($p<0.05$) increased available phosphorus compared to the control treatments. Poultry manure(30t ha^{-1}) treatments significantly ($P<0.05$) enhanced plant height, leaf number and leaf area in both cultivars after 6 months of growth as compared to all other treatments. Under the same treatments of poultry manure (30t ha^{-1}) the white cultivar plants recorded the most significant mean yield parameters after 9 months of growth. The average tuber number per plant (8), tuber weight (250g), tuber length (14cm) and tuber girth (18.5cm) compared to the red cultivar average yield parameters: tuber number per plant (5.7), tuber weight (124.7g), tuber length (8cm) and tuber girth (14.5cm). Biochemically the application of various treatments of poultry manure and NPK fertilizers significantly ($P<0.05$) influenced the changes observed in the secondary metabolites studied. Two months after planting, NPK (150kg ha^{-1}) treatments showed the most significant phenolic content (1.22±0.13mg eq catechin.g^{-1} FW) and flavonoid content (1.08±0.16mg g^{-1} FW) in white cv cocoyam plants. After 6 months of growth, the poultry manure (30t ha^{-1}) treatments recorded the most significant protein content (5.04±0.38mg eq BSA.g^{-1} FW) and peroxidase activity (4.89±1.36 UE min^{-1}) in white cv cocoyam plants while red cv cocoyam plants had the most significant glucanase activity (9.33±1.17 mg eq glucose g^{-1} FW). NPK (150kg ha^{-1}) treatments in white cv cocoyam plants recorded the most significant polyphenol oxidase activity (4.99±0.10 in D330 nm^{-1} min^{-1} UE^{-1} g^{-1} FW) 6 months after planting.

Keywords: *Xanthosoma sagittifolium* L. Schott; Minitubers; cation exchange capacity; Poultry manure; NPK fertilizers; yield parameters and secondary metabolites.

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1. Introduction

Cocoyam (*Xanthosoma sagittifolium* L. Schott, Araceae) is a herbaceous plant cultivated in tropical and subtropical regions for its edible tubers and leaves. Cocoyam tubers and leaves are essentially rich in sugars, proteins, vitamins and mineral salts (Sefa & Sackey, 2004). It covers the food needs of more than 200 million persons in the tropical and subtropical regions and more than 400 million persons worldwide (Onokpise *et al.,* 1999). It occupies the sixth position worldwide with an annual production of 0.45 million metric tonnes (FAO stat, 2006) and the second in Cameroon after cassava (*Mannihot esculenta*) in terms of tuber production. The National Programme on Roots and Tubers (NPRT) was launched in Cameroon in 2005. Annual production of cocoyam has since increased from 1,240,037 tons in 2005 to 2,000,000 tons (NPRT stat, 2013) with projections of 3000,000 tons by 2020.

In Cameroon, the unavailability of healthy seeds to farmers has affected the National production rate. The infestation of seeds is due to several viral, fungal and bacterial diseases (FAO stat, 2008;Chen & Adams, 2001;Perneel *et al.,* 2006).Otherwise, the lack of cocoyam seeds can be justified by the fact that cocoyam usually

propagates vegetatively from tuber fragments, this increases the dissemination of many pathogens which cause rot disease in cocoyam such as *Pythium myriotylum* (Perneel *et al.,* 2006;Boudjeko *et al.,* 2005) ,*Fusarium oxysporum* and *Fusarium solani* (Ubalua *et al.,* 2008;Anele & Nwawuisi, 2008) and Dasheen mosaic virus that is found in the leaves, corm and cormels (Chen & Adams, 2001). To overcome such problems, efforts have been made to improve the application of tissue culture technology to cocoyam production through *in vitro* regeneration of cocoyam plants using biotechnology (Omokolo *et al.,* 2003).Nowadays the production of cocoyam minitubers can be considered as a revolution like the production of potato minitubers. Cocoyam minitubers could be used as an alternative for basic seeds produced through plantlets obtained *in vitro* (Djeuani *et al.,* 2014).

Inspite of the important role played by cocoyam in feeding, another major factor which accounts for low production is the increasing decline in soil fertility levels and lack of soil management practices for continuous cocoyam cultivation(Agbede *et al.,* 2014). Low activity clays characterize tropical soils and the magnitude of nutrient depletion especially in Africa's agricultural production systems is enormous(Stoorvogel, 1990). This depletion of soil fertility is widely recognized as the major cause of low food crop production in sub-Saharan Africa (Sanchez & Jama, 2000). External fertilising agents to agricultural production systems include mineral fertilisers such as urea, NPK, ammonium nitrate, sulfates, and phosphates; organic fertilisers such as animal manures, composts, and biosolids. The use of mineral fertilizers in sub-Saharan Africa is limited by the lack of purchasing power and scarcity of the product in the smallholder sectors while their continuous use can also lead to a decline in soil organic manure (SOM) by enhancing its decomposition (Giller *et al.,* 2009), making SOM a critical nutrient source. The use of organic and mineral fertilizers are the two major and common ways in which soils are managed since the extinction of shifting cultivation as well as reduction in bush fallow periods (Makinde *et al.,* 2011). The impact of increased use of mineral fertilizers on crops has been high but the resulting soil physical degradation, increased soil acidity and soil nutrient imbalance, resulting in reduced crop yield (Ojeniyi, 2000;Mbah & Mbagwu, 2006) escalating cost and unavailability of mineral fertilizers (Surge *et al.,* 2011) have drawn the attention of researchers back to the use of organic manures. Organic manures contain humic substances which play a vital role in soil fertility and plant nutrition. Plants grown on soils which contain adequate humin, humic adds (HAs), and fulvic adds (FAs) are less subject to stress, are healthier, produce higher yields; and the nutritional quality of harvested foods and feeds are superior. These organic manures like poultry manure are cheaper, readily available and affordable for soil fertility management and improvement in crop yield. The availability of inorganic nitrogen in particular has the potential to influence the synthesis of secondary plant metabolites, proteins, and soluble solids(Baneerjee & Mondal, 2012). Different manures (fish, pigeon and cow) and synthetic fertilizers (nitrogen) have been shown to influence some biochemical activities like phenolic constituents of plants (Tugba *et al.,* 2014).The quantity of phenolic compounds present in a given species of plant material varies with a number of factors such as cultivar, environmental conditions, cultural practices postharvest storage and processing (Chandrasekara & Kumar, 2016). Cocoyam responds very well to input of fertilizer whether organic or inorganic as reported by several workers (Hota *et al.,* 2014;Ogbonna & Nwaeze, 2012;Ojeniyi *et al.,* 2013). It has a high requirement for potassium like all other tuber crops (Obigbesan, 1980). In tuber crops, potassium plays a vital role in the movement of sugars produced in the leaf by photosynthesis to the tubers where the sugars are converted to starch (Abd El Latif *et al.,* 2011) Potatoes require high amounts of potassium (K) and nitrogen (N) fertilizers for optimum growth, production and tuber quality (Al- Moshileh *et al.,* 2005).

Against the above background, this study was carried out to: (1) Determine the seed potential of the white and red cultivar cocoyam (*Xanthosoma sagittifolium* L. Schott**)** minitubers under different fertilizer treatments (Poultry manure and NPK 20:10:10) (2) Evaluate the effects of these fertilizer treatments on the changes in the content of some secondary metabolites in the leaves of white and red cocoyam (*Xanthosoma sagittifolium)* cultivars during growth.

2. Materials and methods

2.1. Site location

A field trial was conducted to study the performance of Xanthosoma sagittifolium minituber seeds as influenced by poultry manure and NPK fertilizer during the 2017 cropping season on an experimental farm at Ngog Bibega, Mbankomo Sub-division, (Outskirsts of Yaoundé) Centre region, Cameroon, located at latitude 3°49′52.54″N and longitude 11°27′15.79″E and 714 m above sea level. The area is characterized as a humid rainforest zone and the soil is clay loam. The total annual rainfalls for 2017 was 1902.8 mm while the total rainfalls during the period of experimentation (April to December) for 2017 was 1775.6 mm.

2.2. Materials

The planting material consisted of white and red cultivars of X. sagittifolium minituber seeds of mean weight 38g produced from acclimatised vitroplants under the shed in the plant physiology Laboratory of the Higher

Teachers Training college (HTTC), University of Yaoundé I. Yaoundé, Cameroon (Djeuani et al., 2014, 2017). The Poultry manure was obtained from Henri et Freres Poultry farm Yaoundé, Cameroon while the NPK fertilizer (20:10:10) was obtained from the fertilizer unit of the Centre Regional Delegation of Agriculture and Rural Development, Yaoundé, Cameroon.

2.3. Experimental design, treatments, soil and morphological analysis during growth and yield of cocoyam plants

The experiment was a 4×2 factorial arrangement in a randomized complete block design and replicated three times. The site was ploughed, ridged and marked out into two main blocks, one for each cultivar. Each of these main blocks was further subdivided into 3 sub blocks which represent the three replicates. Each sub block was divided into five experimental plots, thus a total of fifteen plots were used for cultivar. Each gross plot measured 4m×3m (12 m^2) with a net plot of 2m×2m. Soil samples were collected with soil auger at a depth of 0 to 20 cm from different locations of the site and bulked into composite sample. The composite soil sample was air dried, passed through 2 mm sieve, and then analyzed for its physicochemical properties before planting and after harvest (Table1). Particle size distribution was performed to determine the soil textural class using hydrometer method as described by Gee & Bauder, 1986. Soil pH was determined in distilled water and potassium chloride solution using pH meter (Mclean, 1982). Soil nitrogen was determined by Macro-Kjeldahl digestion method (Bremner, 1982). Exchangeable Ca and Mg were obtained by the complexometric titration method of Chapman, 1982, and exchangeable Na and K were determined by flame photometer. Cation Exchange Capacity (CEC) was determined by modified ammonium acetate method of Chapman, 1982 while available P determined by Bray II method (Bray, 1945). The treatments comprised three rates each of application of Poultry Manure (0, 20, and 30 t ha-1) and NPK fertilizer (0, 120, and 150 kg ha-1). A total of fifteen treatment combinations and three replications were used. The Poultry manure was incorporated into the soils on the experimental plots in a single application based on the treatment combinations, at 2 weeks before planting to ease decomposition, while the NPK fertilizer was applied to the cocoyam stands according to treatment allocation at 4 weeks after planting (WAP) using the ring placement method. Each minituber was planted per hole at a depth of 15 cm and at a spacing of 0.5 m x 1.0m resulting to about twenty-five plants per plot and a total of 375 plants per cultivar. All plots were kept weed free by manual weeding. Five cocoyam plants were randomly selected from each of the net plots, tagged and then used for the determination of average plant height (cm), average number of leaves, and average leaf area at 1, 2, 3,4,5,6,7, and 8 months after planting (MAP). Yield parameters like length (cm) and girth (cm) of tubers, number of tubers per plant, and tuber weight (g) were assessed after 9 months at physiological maturity. The leaf area was determined using the formula of (Biradar, 1982) as: Leaf Area of Cocoyam = 0.917 (LW). Where L and W are length and width of the cocoyam leaf.

2.4. Biochemical analysis

Biochemical analyses consisted of extraction and assay of total soluble proteins, peroxidases, β-1,3 glucanases, Polyphenoloxidases, Total Phenol content and flavonoid content in the leaves each month for 6 months after planting.

2.4.1 Protein Content

Proteins were extracted according to the modified method of Pirovani, 2008. 1g of leaves were ground in chilled mortar with 5 mL of Tris- Maleate buffer (10mM pH 7.2). The crude homogenate was centrifuged for 25 min at 10000 g and 4°C after incubation. The supernatant was removed and used as crude extract for protein and enzymes assays. Proteins were quantified according to method described by Bradford, 1976. 10 µl of extract were added to 490 µl of distilled water and 500 µl of Bradford reagent. The mixture was incubated at 25°C in darkness for 15 min and OD of protein determined at 595nm. The protein content was expressed in mg-equivalent of BSA per Fresh Weight (Bradford, 1976).

2.4.2 Peroxidase Assay

Peroxidase assay in protein extract was done according the modified method of Baaziz et al., 1994. The reaction medium contained 925 µl of Tris- Maleate buffer (10mM pH 7.2, containing 1g gaïacol), 25 µl of protein extract and 50 µl of H_2O_2 (10%). The mixture was incubated for 3min at 25°C. Peroxidase activity was determined following the formation of tetragaïacol at 470nm. Peroxidase activity was expressed in enzyme units per Fresh Weight (FW).

2.4.3. β-1,3 Glucanase Assay

The activity of β-1,3-glucanases was evaluated in the protein extract according to the modified method described by Leelasuphakul, 2006. The reaction mixture contained 90 µl of sodium acetate buffer (0.1M, pH 4 containing 25mg/L of Laminarin), 10 µl of protein extract incubated for 10 min at 40°C. 200 µl of 2M HCl is used to stop the reaction. The OD was read at 540nm. β-1,3-glucanase activity was expressed in µmole of glucose released/min/g of Fresh Weight.

2.4.4. Polyphenol oxidase Assay

Polyphenol oxidase assay was determined in the protein extract according to the method of Vankammenn & Broumer, 1964. The reaction mixture contained 500 µl of phosphate buffer (0.66M, pH 7), 150 µl of catechine and 35 µl of protein extract incubated at 25°C for 30 s. The change in absorbance was read after 5min at 330nm. Polyphenol oxidase activity was expressed in D_{330nm} /min/UE/g of Fresh Weight (FW)

2.4.5. Total Phenol Content

Phenolic compounds were extracted according to the modified method of Boudjeko et al., 2007. 1 g of leaves of cocoyam was ground in chilled mortars with 5 ml of 80% (v/v) methanol at 4°C. After incubation, tubes were centrifuged thrice at 7000 g for 30 min, supernatants were collected each time. Mixture of the three supernatants constituted the crude extract. Total phenols were quantified using the method described by Marigo, 1973. 10 µl of alcoholic extract were added to 500 µl of distilled water, Folin-Ciocalteu reagent (75 µl) and 500 µl of sodium carbonate (20%). The mixture was incubated at 40°C for 20 min and the blue color was determined at 760 nm. The content of soluble phenolic was expressed in mg-equivalent of gallic acid per Fresh Weight (FW).

2.4.6. Flavonoid Content

Flavonoid content was determined according to the modified method of Kramling, 1969.The reaction medium contains 400 µl of phenol extract, 200µl of HCl (50%), 200 µl of Formaldehyde (8mg/L) incubated at 4°C for 15min. The mixture was centrifuged at 3000 g for 5min and the supernatant collected. The supernatant was then used to assay non flavonoid compounds according to the method described by Marigo, 1973. Flavonoid content was then determined using the following formula:

$$T_{flavonoids} = T_{\text{total phenol}} - T_{\text{non flavonoids}}$$

3. Results

3.1. Soil analyses before planting and after harvest

Physical analysis of the sand, clay and silt content of the soil on the different treatment plots before planting and after harvest on our experimental farm illustrated a clay loam textural class. (Table 1). Chemically, before planting the soil was slightly acidic (pH 5.6) and both poultry manure treatments (20t.ha^{-1} and 30t ha^{-1}) after harvest significantly reduced soil acidity (pH 6.6 and pH 6.9) (Table 2). Poultry manure treatments (20t ha^{-1} and 30t ha^{-1}) significantly increased organic and carbon content and also recorded the highest cation exchange capacity values as compared to the other treatments. (Table 2).

3.2. Evaluation of the growth parameters of X. sagittifolium plants under different treatments

All growth parameters (plant height, number of leaves plant^{-1} and leaf area) analysed were generally more significant in the white cultivar cocoyam (white cv) plants than in the red cultivar cocoyam (red cv) plants for all treatments. The poultry manure treatments (30t ha^{-1}) showed the most significant increase in all growth parameters analysed from two months after planting to six months after planting among all treatments meanwhile within the same growth period the control treatments (0t of Poultry manure ha^{-1} and 0kg of NPK ha^{-1}) recorded the least increase in average plant height and leaf area for both white and red cultivars of the cocoyam plants. At six months after planting white cv cocoyam plants treated poultry manure (30t ha^{-1}) showed the most significant growth in terms of average height (86cm) (Table 3, Fig 1.) followed by an average height of 77cm for cv red cocoyam plants treated with poultry manure(30tons/ha). (Table 4, Fig 1.). The cv red cocoyam plants showed no significant difference in the average number of leaves for all the different treatments throughout the growth period (Table 4.) meanwhile a significant average number of leaves was recorded (6) after six months of growth for white cv cocoyam plants treated with poultry manure (30t ha^{-1}) (Table 3). White cv plants treated with poultry manure (30t ha^{-1}) after six months of growth recorded the most significant average leaf area (0.88m^2) (Table 3) while red cv cocoyam plants treated with poultry manure (30t.ha^{-1}) recorded an average leaf area of 0.069m^2 after six months of growth. (Table 4)

3.3. Evaluation of yield parameters of cocoyam under different treatments

After a period of 9 months the yield parameters were assessed. The four yield parameters (tuber number plant^{-1}, tuber weight, tuber length and tuber girth) analysed after harvest were generally greater in white cv cocoyam plants than in red cv cocoyam plants for all treatments. Poultry manure treatments (30t ha^{-1}) showed the most significant yield parameters while the control treatments (0tons of Poultry manure ha^{-1} and 0kg of NPK ha^{-1}) had the least yield parameters for both cultivars. White cv plants treated with poultry manure (30t ha^{-1}) recorded an average tuber number per plant of 8, an average tuber weight of 250g, an average tuber length of 14cm, and an average girth of 18.5cm (Table 5 and Fig 2) meanwhile red cv plants treated with poultry manure (30t ha^{-1}) recorded 5.7 as average tuber number per plant, 124.7g as average tuber weight, 8cm as average tuber length and 14.5cm as average tuber girth.(Table 6 and Fig 2).

3.4. Evaluation of some biochemical parameters

After 6 months of growth, the treatment poultry manure (30 t.ha^{-1}) recorded the most significant protein content (5.04±0.38mg eq BSA g^{-1} FW) for white cv cocoyam plants followed by the same treatments for red cv (4.11±0.52mg eq BSA g^{-1} FW). The Control White cv plants (0 tons of Poultry manure ha^{-1} and 0 kg of NPK ha^{-1}) had the least protein content (2.56±0.29 mg eq BSA g^{-1} FW) while there was no significant difference for the Control and NPK (150kg ha^{-1}) treatments in red cv plants after 6 months of growth. (Fig.3A).

Peroxidase activity increased in the three treatments (Control, Poultry manure 30t ha^{-1} and NPK 150kg ha^{-1}) for white cv plants from 2 months after planting to six months after planting with Poultry manure 30t ha^{-1} recording the most significant value of 4.89±1.36 UE min^{-1} at 6 months. The Control, and NPK 150kg ha^{-1} treatments for red cv plants recorded an increase in peroxidise activity between 2 and 4 months after planting, followed by a significant decrease at 6 months (Fig.3B)

The most significant value of glucanase activity was observed in red cv cocoyam plants treated with Poultry manure (30t ha^{-1}), 9.33±1.17 mg eq glucose g^{-1} FW meanwhile white cv cocoyam plants treated with Poultry manure (30t ha^{-1}), recorded 7.36±0.28mg eq glucose g^{-1} FW after six months of planting. The least value for glucanase activity was seen in red cv plants treated with NPK (150kg ha^{-1}), 1.34±.24 mg eq glucose g^{-1} FW after 4 months of planting (Fig.4A).

After 6 months of growth, polyphenol oxidase activity was most significant in Cv white cocoyam plants treated with NPK (150kg ha^{-1}), 4.99±0.10 in D330 nm^{-1} min^{-1}UE^{-1}g^{-1} FW while the red cv Control, Poultry manure (30t ha^{-1}) and NPK (150kg ha^{-1}) treatments showed no significant difference in polyphenol oxidase Red cv cocoyam plants treated with Poultry manure (30t ha^{-1}) recorded a polyphenol oxidase activity of 4.39±0.33 in D330 nm^{-1} min^{-1} UE^{-1} g^{-1} FW after 4 months of planting (Fig.4B).

NPK (150kg ha^{-1}) treatments recorded the most significant values in phenol contents in both cultivars, white cv cocoyam plants had 1.22±0.13 mg eq catechin g^{-1} FW and red cv cocoyam plants had 1.12±0.11mg eq catechin g^{-1} FW after 2 months of planting. The white cv cocoyam plants showed no significant difference in phenolic contents in the Control, Poultry manure (30t ha^{-1}) and NPK (150kg ha^{-1}) treatments after 4 months of planting (Fig.5A).

Flavonoid content was greatest at 2 months after planting in red cv cocoyam plants treated with NPK (150kg ha^{-1}), 1.08±0.16mg g^{-1} FW while white cv cocoyam plants treated with Poultry manure (30t ha^{-1}) also recorded a significant flavonoid content value of 0.96±0.03mg g^{-1} FW after 6 months of growth. The least flavonoid content value was recorded by the control red cv cocoyam plants, 0.06±0.01 mg g^{-1} FW after 6months of growth (Fig 5B)

Table 1. Physical analyses of soil before planting and after harvest

Physical properties (%)	**Farm before planting**	**Treatments plots after harvest**				
		Control	Poultry manure (20t ha^{-1})	Poultry manure (30t ha^{-1})	NPK (120kg ha^{-1})	NPK (150kg ha^{-1})
Moisture content	3.47	3.07	4.38	5.39	3.72	3.77
Sand	29.3	28.3	29.8	29.1	29.2	28.8
Silt	35.6	35.5	34.8	35.3	35.6	35.4
Clay	35.0	36.1	35.3	35.5	35.1	35.7
Textural class	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam

Table 2. Chemical analyses of soil before planting and after harvest

Chemical properties	Farm before planting	Treatments plots after harvest				
		Control	Poultry manure (20t.ha^{-1})	Poultry manure (30t ha^{-1})	NPK (120kg ha^{-1})	NPK (150kg ha^{-1})
pH in Water	5.6	5.5	6.6	6.9	5.6	5.4
pH in KCl	4.4	4.2	6.4	6.8	4.7	4.4
Organic matter (g/kg)	23.44	21.75	48.31	55.85	23.41	21.49
Total N (g/kg)	1.1	1.1	1.4	1.7	1.5	1.6
Total C (g/kg)	13.44	12.61	28.02	32.40	13.73	12.26
Available P (mg/kg)	4.57	3.91	57.84	94.04	113.5	129.9
Exchangeable Ca (cmol/kg)	1.6	1.62	1.96	2.39	1.91	1.99
Exchangeable Mg (cmol/kg)	0.75	0.69	0.81	1.03	0.75	0.81
Exchangeable K (cmol/kg)	0.08	0.09	0.14	0.29	0.19	0.23
Exchangeable Na (cmol/kg)	0.10	0.10	0.49	0.53	0.21	0.31
Cation exchange capacity (cmol/kg)	5.0	5.0	6.0	6.0	5.6	5.6

Table 3. Growth parameters of white cv *X. sagittifolium* plants

Treatment	Average plant height (cm)				Average number of leaves				Average leaf area (m^2)			
	2map	4map	6map	8map	2map	4map	6map	8map	2map	4map	6map	8map
Control	35^e	42^e	55^e	25^e	2^a	3^{ab}	2^c	1^a	0.031^d	0.021^e	0.025^e	0.002^e
PM1	70^b	73.7^b	77^b	55^b	3^a	4^{ab}	5^{ab}	2^a	0.042^b	0.053^b	0.072^b	0.021^d
PM2	80^a	86^a	86^a	62^a	4^a	5^a	6^a	3^a	0.051^a	0.067^a	0.088^a	0.031^a
NPK1	40^d	47.7^d	60^d	39^d	2^a	2^b	3^{bc}	1^a	0.033^d	0.042^d	0.056^d	0.025^c
NPK2	50^c	60^c	70^c	45^c	4^a	4^a	3^{bc}	1^a	0.039^c	0.048^c	0.061^c	0.028^b
Significance	*	*	*	*	NS	*	*	NS	*	*	*	*

KEY: map= months after planting, PM1= Poultry manure (20t ha^{-1}), PM2= Poultry manure (30t ha^{-1}), NPK1= NPK fertilizer (120Kg ha^{-1}), NPK2=NPK fertilizer (150kg ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability.

Table 4. Growth parameters of red cv *X. sagittifolium* plants

Treatment	Average plant height (cm)				Average number of leaves				Average leaf area (m^2)			
	2map	4map	6map	8map	2map	4map	6map	8map	2map	4map	6map	8map
Control	23^d	41^e	50^e	40^e	2^a	3^a	4^a	1.5^a	0.020^c	0.031^c	0.041^c	0.018^c
PM1	29^b	51^b	60^b	38^c	3^a	3^a	5^a	2^a	0.027^b	0.043^b	0.065^b	0.021^b
PM2	35^a	62^a	77^a	59^a	4^a	4.3^a	5^a	3^a	0.034^a	0.054^a	0.069^a	0.031^a
NPK1	26^c	46^d	55^d	38^c	2^a	2.7^b	4^a	1.3^a	0.022^c	0.028^d	0.041^d	0.011^d
NPK2	30^b	48^c	58^c	51^b	3^a	3.3^a	4.5^a	1.6^a	0.025^b	0.041^c	0.058^c	0.013^d
Significance	*	*	*	*	NS	NS	NS	NS	*	*	*	*

KEY: map= months after planting, PM1= Poultry manure (20t ha^{-1}), PM2= Poultry manure (30t ha^{-1}), NPK1= NPK fertilizer (120Kg ha^{-1}), NPK2=NPK fertilizer (150kg ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability .

Table 5. Yield parameters of white cv *X. sagittifolium* plants.

Yield Parameters	TREATMENTS					Significance
	Control	PM1	PM2	NPK1	NPK2	
Tuber number plant^{-}	2 ^{c}	4^{bc}	8^a	3^c	6^{ab}	*
Tuber Weight (g)	20^e	100^c	250^a	80^d	230^b	*
Tuber Length (cm)	6^c	10^b	14^a	7^c	12^{ab}	*
Tuber Girth (cm)	10.5^c	13.5^b	18.5^a	11.5^{bc}	17^a	*

KEY: PM1= Poultry manure (20t.ha^{-1}), PM2= Poultry manure (30t.ha^{-1}), NPK1= NPK fertilizer (120Kg.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability.

Table 6. Yield parameters of red cv *X. sagittifolium* plants

Yield Parameters	TREATMENTS					Significance
	Control	PM1	PM2	NPK1	NPK2	
Tuber number plant^{-1}	2.3 ^{c}	4^{b}	5.7^{a}	2.7^{bc}	3.6^{bc}	*
Tuber Weight (g)	22^{e}	52^{c}	124.7^{a}	42^{d}	94^{b}	*
Tuber Length (cm)	3^{b}	5^{b}	8^{a}	5^{b}	8^{a}	*
Tuber Girth (cm)	6^{c}	7^{b}	14.5^{a}	8^{b}	13^{a}	*

KEY: PM1= Poultry manure (20t ha^{-1}), PM2= Poultry manure (30t ha^{-1}), NPK1= NPK fertilizer (120Kg ha^{-1}), NPK2=NPK fertilizer (150kg ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability.

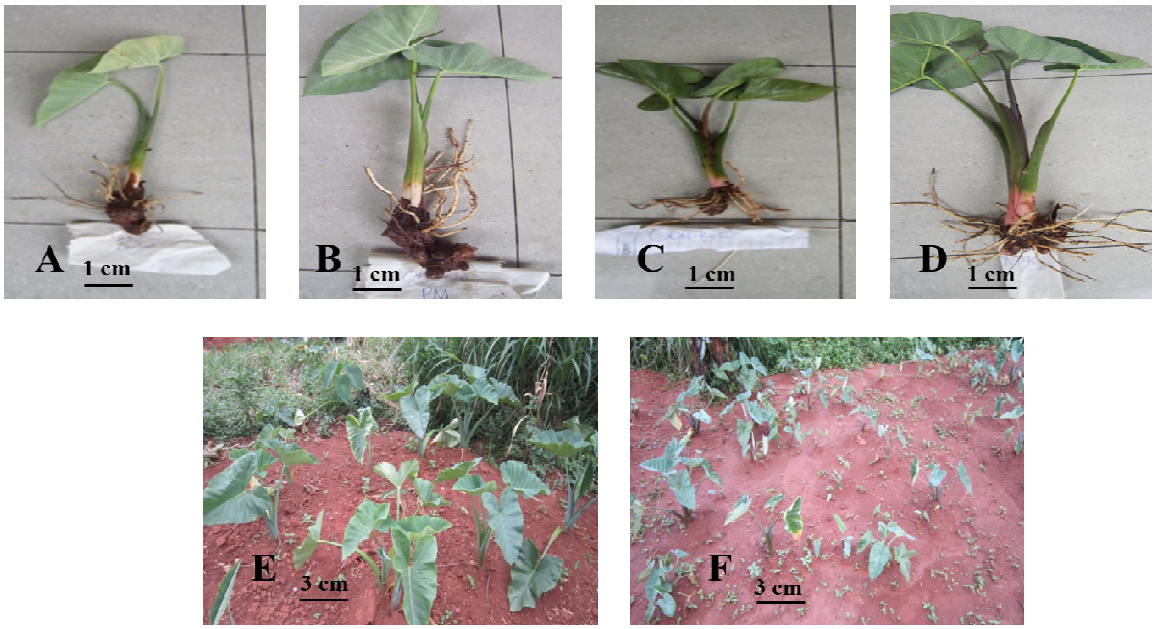

Fig.1. Aspect of cocoyam plant treatments after 6 months of growth: white cv control (A); white cv poultry manure (30tons/ha) (B); red cv control (C); red cv poultry manure (30 tons/ha) (D); white cv plot poultry manure (30tons/ha) (E); red cv plot poultry manure (30tons/ha)(F).

Fig.2. Aspect of cocoyam plant treatments at harvest after 9 months: white cv control (A); white cv poultry manure (30tons/ha) (B); red cv control (C); red cv poultry manure (30 tons/ha) (D); white cv tubers poultry manure (30tons/ha) (E); red cv tubers poultry manure (30tons/ha) (F); girth difference of white and red cvs (G). Dead leaves (L), Rhizomes (Rh) and tubers (t),

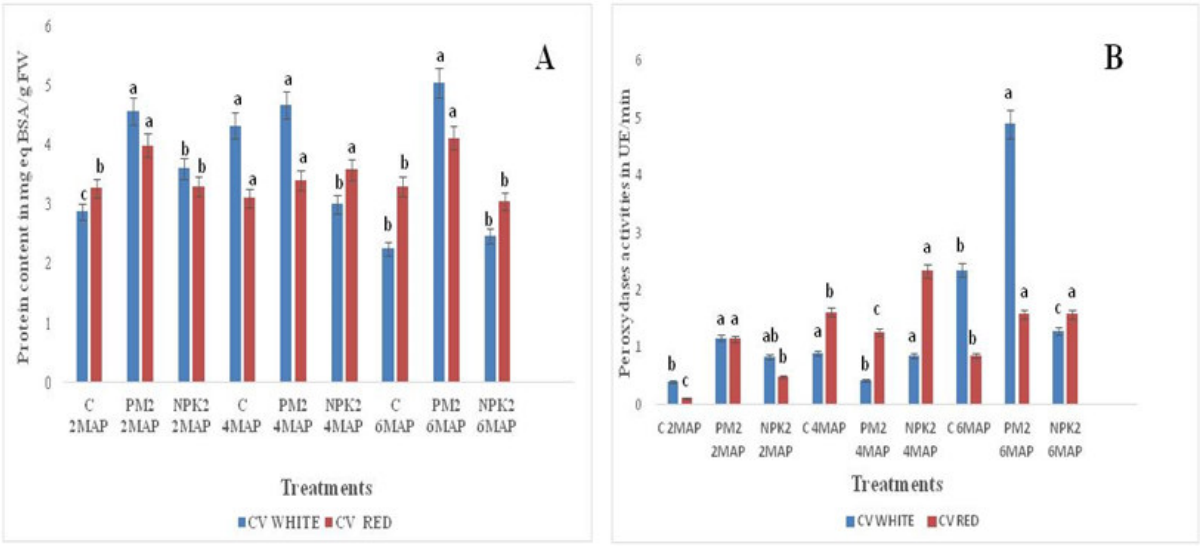

Fig.3. Protein content (mg eqBSA/FW) (A) and Peroxidase activities (UE/min) (B) in white and red cv cocoyam leaves during growth under different treatments. KEY: PM1= Poultry manure (20tons.ha^{-1}), PM2= Poultry manure (30tons/ha), NPK1= NPK fertilizer (120Kg.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), MAP= months after planting.

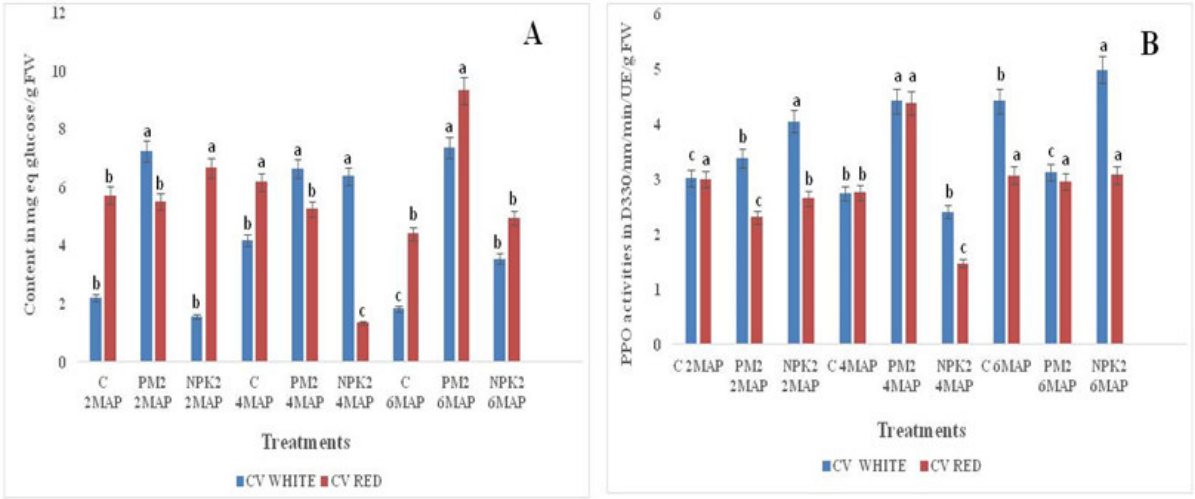

Fig.4. Glucanase activities (mg eq glucose.g^{-1} FW) (A) and Polyphenol oxidase activities (D330/nm/min/UE/g FW) (B) in white and red cv cocoyam leaves during growth under different treatments. KEY: PM1= Poultry manure (20tons.ha^{-1}), PM2= Poultry manure (30tons/ha), NPK1= NPK fertilizer (120Kg.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), MAP= months after planting.

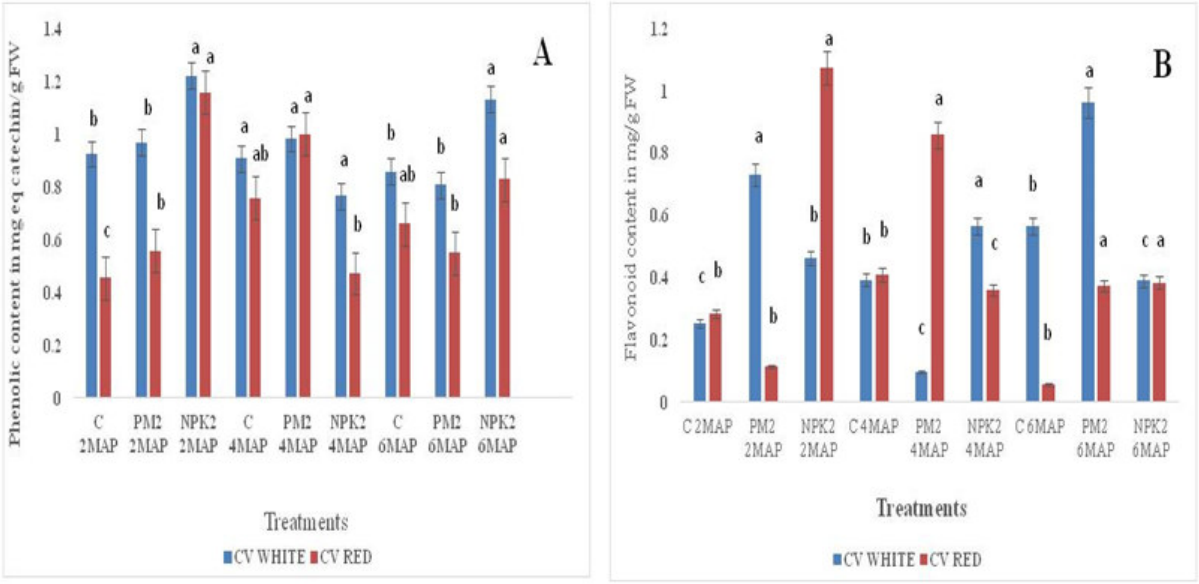

Fig.5. Phenolic content (mg eq catechin/g FW) (A) and in Flavonoid content (mg.g^{-1} FW) (B) in white and red cv cocoyam leaves during growth under different treatments. KEY: PM1= Poultry manure (20tons.ha^{-1}), PM2= Poultry manure (30tons/ha), NPK1= NPK fertilizer (120Kg.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), MAP= months after planting.

4. DISCUSSION

Effects of poultry manure and NPK fertilizer treatments on growth and yield parameters of the white and red cultivars of cocoyam (*Xanthosoma sagittifolium* L. Schott) minituber seeds were assessed. The growth and yield performance for the white cultivar cocoyam plants were significantly higher than those for the red cultivar cocoyam plants. These results are concordant with those obtained by Nzietchueng, 1985 who evaluated the production problems faced by different cocoyam (*Xanthosoma*) cultivars and showed that the white cultivar was more productive than the red cultivar. As observed from the results, it was evidenced that poultry manure (30 t ha^{-1}) treatments significantly produced the highest means of traits assessed, while the control treatments of no poultry manure or no NPK fertilizer applied significantly produced the lowest means of the same traits over a 9 month growth and yield period in both cultivars.150 kg ha^{-1} NPK fertilizer treatments expressed the most significant means of traits among all the NPK fertilizer treatments in both cultivars. Similar observations were earlier reported by Hamma *et al.,* 2014, when they evaluated the performance of cocoyam (*Colocasia esculentus.L*) under the influence of organic and inorganic manure in Samaru, Zaria, Nigeria. They observed that poultry manure (10 t ha^{-1}) produced the most significant means of growth and yield parameters among treatments of no manure (control), goat manure (10 t ha^{-1}) and cow manure (10 t ha^{-1}). They also showed that 150 kg ha^{-1} NPK fertilizer treatments produced the most significant means of growth and yield traits among no NPK fertilizer (control), 90 kg NPK ha^{-1} and120 kg NPK ha^{-1} fertilizer treatments.

The fact that these results depicted better crop performance in all parameters measured with poultry manure (30t ha^{-1}) than in all NPK fertilizer treatments for both white cv and red cv could be attributed to the fact that poultry manure treatments had a more favourable influence on soil pH, and soil organic content by increasing them and recorded higher cation exchange capacity values than all other treatments. Similar results were obtained by Gülsüm *et al.,* 2019 who showed that poutry manure applications improved soil organic matter content, exchangeable cations, cation exchange capacity, and percent base saturation thereby enhancing yield of sweet basil (*Ocimum basilicum* L.).Therefore poultry manure treatment plots retained more nutrients in the soil than the other treatments and slowly released these nutrients to the plants with reduced leaching losses of nutrients throughout the growth period Uwah *et al.,* 2011. The chemical composition of the different treatment plots after harvest (Table2.) showed that those treated with poultry manure (30 t ha^{-1}) had a higher N, K and organic carbon content which could also account for better growth and yield performance. These results are similar to those obtained by Uwah *et al.,* 2011 , who evaluated the effect of organic and mineral fertilizers on growth and yield of taro (*Colocasia esculenta* (L.) Schott and showed that when the N,P,K and Ca contents of poultry manure increased on a site, it led to superior crop performance. Concordant results have also been obtained by Karamat *et al.,* 2019 who showed that poultry litter had a significant increase in grain yield and N,P,K uptake by corn (*Zea mays*).

Protein content was most significant in white cv plants treated with poultry manure (30 t ha^{-1}) after 6 months of growth. These results are contrary to those obtained by Borgmann *et al.,* 1994 who showed that the

total protein content decreased during the maturation of microtubers in irish potatoes. Since proteins play an important role in the growth and repair of plant cells, this could suggest a positive correlation between protein content and the different growth parameters assessed during growth of plants treated with Poultry manure (30t ha^{-1}). The control and NPK2 treatments of both cultivars recorded a decrease in protein content between 4months and 6months after planting (Fig.3), corroborating results obtained by Djeuani, 2017 obtained a decrease in leaf protein content over time especially during maturation of cocoyam minitubers.

Biotic or abiotic stress may account for the significant increase in peroxidise activity noticed in the white cv plants treated with Poultry manure (30 $t.ha^{-1}$) and the during the first 6 months of growth. These results are similar with those obtained by Djocgoue,1998 ; Baaziz *et al.,* 2006;Mas & Heng, 2019, who showed that generally, in case of a wound, microbial infection or any other unfavourable condition, new isoperoxidases appear.

Our results also illustrate very significant levels of glucanase activities in both cultivars treated with poultry manure (30 t ha^{-1}) after 6 months of growth. Hereby suggesting roles of glucanases in plant stress relief and agreeing with Vaiyapuri *et al.,* 2012 who evidenced that β 1,3 glucanases play key roles in cell division, impeding cell to cell virus movements in plants by regulating callose turnover at plasmodesmata, and withstanding abiotic stress and Xiaohui *et al.,* 2019 who revealed the involvement of gluanases in fungal growth inhibition mechanisms by the rhizobacteria (*Paenibacillus jamilae* HS-26).

Polyphenol oxidases(PPOs) catalyse the oxidation of phenolic compounds into highly reactive quinones. Polyphenol oxidase activities were significant in white cv plants after 6 months of growth and in red cv plants after 4 months of growth both treated with NPK_2 (150 kg ha^{-1}). These results agree with Steffens *et al.,* 1994 who suggested that *in vivo* polyphenol oxidase activity can be associated to senescing, wounding or damage to plant tissues in which cellular compartmentalization is lost. This is justified by the fact that classically polyphenol oxidases and their potential phenolic substrates are physically separated from one another in plants. Polyphenol oxidases are found in chloroplasts while phenolic compounds are found primarily in the vacuole and cell wall (Vaughn *et al.,* 1998). Similarly, Ioannis *et al.,* 2019 had results which indicated that PPOs could accept flavonoids as their natural substrates and therefore might participate in the synthetic pathways of secondary metabolites.

At 2 months after planting both cultivars treated with NPK_2 (150 kg ha^{-1}) recorded the most significant phenolic content values. NPK_2 may have increased soil salinity, leading to the formation of large amounts of oxygen free radicals (O_2•-) in peroxisomes. Accumulation of ROS results in “oxidative stress”. ROS initiate free radical reactions that lead to oxidation of proteins, lipids and nucleic acids impairing their functions and causing cell death. This therefore justifies an increase in phenolic contents which form an antioxidant system developed by plants to counter “oxidative stress” by capturing ROS (Gill & Tutejn, 2010;Sharma *et al.,* 2012). Mas & Heng, 2019 also depicted that total phenolic content in the extracts of *Pereskia bleo* leaves significantly influenced antioxidant and antimicrobial activities.

Significant flavonoid content values were recorded 6 months after planting in white cv plants and 4 months after planting in red cv plants both treated with poultry manure (30 t/ha^{-1}). Flavonoids participate in plant protection against biotic (herbivores, microorganisms) and abiotic stresses (UV radiation, heat), and due to their antioxidative properties, they also maintain a redox state in cells. The antioxidative activity of flavonoids is connected with the structure of the molecule: the presence of conjugated double bonds and the occurrence of functional groups in the rings (Amić, et al., 2003;Seyoum *et al.,* 2006;Ireneusz *et al.,* 2018;Wang *et al.,* 2019). Flavonoids reduce the production of and quench reactive oxygen species (ROS) through: suppression of singlet oxygen; inhibition of enzymes that generate ROS (cyclooxygenase, lipoxygenase, monooxygenase, xanthine oxidase); chelating ions of transition metals, which may catalyze ROS production; quenching cascades of free-radical reactions in lipid peroxidation;“re-cycling” of other antioxidants (Rice-Evans *et al.,* 1996;Cotelle *et al.,* 1996;Arora *et al.,* 2000).

5. Conclusion

The objective of this pioneering field trial of *Xanthosoma sagittifolium* (white and red cultivars) minitubers as seeds was to evaluate their performance in response to different treatments of poultry manure and NPK fertilizers. Our results show that for all treatments, the white cultivar *X. sagittifolium* minitubers depicted better growth and yield parameters than the red cultivar minitubers. Poultry manure (30t ha^{-1}) stimulated the most significant responses in terms of better growth and yield among all treatments in both cultivars, suggesting that poultry manure offers a higher nutrient content and a more favourable pH to the soil than the other treatments. Both poultry manure (30 t ha^{-1}) and NPK (150kg ha^{-1}) treatments in both cultivars significantly influenced total soluble protein content, peroxidase activity, glucanase activity, polyphenoloxidase activity, phenolic contents and flavonoid content in the leaves during growth. These secondary metabolites play key roles in controlling abiotic and biotic stress thereby enhancing growth and development in plants. This work serves as an initial step in the improvement of cocoyam (*Xanthosoma sagittifolium*) production in Cameroon using cocoyam minitubers

as seeds.

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Conflicts of Interests
The authors have not declared any conflict of interest

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