ABOUT AJB

The African Journal of Biotechnology (AJB) (ISSN 1684-5315) is published weekly (one volume per year) by Academic Journals.

African Journal of Biotechnology (AJB), a new broad-based journal, is an open access journal that was founded on two key tenets: To publish the most exciting research in all areas of applied biochemistry, industrial microbiology, molecular biology, genomics and proteomics, food and agricultural technologies, and metabolic engineering. Secondly, to provide the most rapid turn-around time possible for reviewing and publishing, and to disseminate the articles freely for teaching and reference purposes. All articles published in AJB are peer-reviewed.

Submission of Manuscript

Please read the Instructions for Authors before submitting your manuscript. The manuscript files should be given the last name of the first author

Click here to Submit manuscripts online

If you have any difficulty using the online submission system, kindly submit via this email ajb@academicjournals.org.

With questions or concerns, please contact the Editorial Office at ajb@academicjournals.org.
Editor-In-Chief

George Nkem Ude, Ph.D
Plant Breeder & Molecular Biologist
Department of Natural Sciences
Crawford Building, Rm 003A
Bowie State University
14000 Jericho Park Road
Bowie, MD 20715, USA

Editor

N. John Tonukari, Ph.D
Department of Biochemistry
Delta State University
PMB 1
Abraka, Nigeria

Associate Editors

Prof. Dr. AE Aboulata
Plant Path. Res. Inst., ARC, POBox 12619, Giza, Egypt
30 D, El-Karama St., Alf Maskan, P.O. Box 1567,
Ain Shams, Cairo,
Egypt

Dr. S.K Das
Department of Applied Chemistry
and Biotechnology, University of Fukui,
Japan

Prof. Okoh, A. I.
Applied and Environmental Microbiology Research
Group (AEMREG),
Department of Biochemistry and Microbiology,
University of Fort Hare.
P/Bag X1314 Alice 5700,
South Africa

Dr. Ismail TURKOGLU
Department of Biology Education,
Education Faculty, Firat University,
Elazığ,
Turkey

Prof T.K.Raja, PhD FRSC (UK)
Department of Biotechnology
PSG COLLEGE OF TECHNOLOGY (Autonomous)
(Affiliated to Anna University)
Coimbatore-641004, Tamilnadu,
INDIA.

Dr. George Edward Mamati
Horticulture Department,
Jomo Kenyatta University of Agriculture
and Technology,
P. O. Box 62000-00200,
Nairobi, Kenya.

Dr. Gitonga
Kenya Agricultural Research Institute,
National Horticultural Research Center,
P.O Box 220,
Thika, Kenya.
Editorial Board

Prof. Sagadevan G. Mundree  
*Department of Molecular and Cell Biology*  
*University of Cape Town*  
*Private Bag Rondebosch 7701*  
*South Africa*

Dr. Martin Fregene  
*Centro Internacional de Agricultura Tropical (CIAT)*  
*Km 17 Cali-Palmira Recta*  
*AA6713, Cali, Colombia*

Prof. O. A. Ogunseiyan  
*Laboratory for Molecular Ecology*  
*Department of Environmental Analysis and Design*  
*University of California,*  
*Irvine, CA 92697-7070. USA*

Dr. Ibrahima Ndoye  
*UCAD, Faculte des Sciences et Techniques*  
*Departement de Biologie Vegetale*  
*BP 5005, Dakar, Senegal.*  
*Laboratoire Commun de Microbiologie*  
*IRD/ISRA/UCAD*  
*BP 1386, Dakar*

Dr. Bamidele A. Iwalokun  
*Biochemistry Department*  
*Lagos State University*  
*P.M.B. 1087. Apapa – Lagos, Nigeria*

Dr. Jacob Hodeba Mignouna  
*Associate Professor, Biotechnology*  
*Virginia State University*  
*Agricultural Research Station Box 9061*  
*Petersburg, VA 23806, USA*

Dr. Bright Ogheneovo Agindotan  
*Plant, Soil and Entomological Sciences Dept*  
*University of Idaho, Moscow*  
*ID 83843, USA*

Dr. A.P. Njukeng  
*Département de Biologie Végétale*  
*Faculté des Sciences*  
*B.P. 67 Dschang*  
*Université de Dschang*  
*Rep. du CAMEROUN*

Dr. E. Olatunde Farombi  
*Drug Metabolism and Toxicology Unit*  
*Department of Biochemistry*  
*University of Ibadan, Ibadan, Nigeria*

Dr. Stephen Bakiamoh  
*Michian Biotechnology Institute International*  
*3900 Collins Road*  
*Lansing, MI 48909, USA*

Dr. N. A. Amusa  
*Institute of Agricultural Research and Training*  
*Obafemi Awolowo University*  
*Moor Plantation, P.M.B 5029, Ibadan, Nigeria*

Dr. Desouky Abd-El-Haleem  
*Environmental Biotechnology Department & Bioprocess Development Department,*  
*Genetic Engineering and Biotechnology Research Institute (GEBRI),*  
*Mubarak City for Scientific Research and Technology Applications,*  
*New Burg-Elarab City, Alexandria, Egypt.*

Dr. Simeon Oloni Kotchoni  
*Department of Plant Molecular Biology*  
*Institute of Botany, Kirschallee 1,*  
*University of Bonn, D-53115 Germany.*

Dr. Eriola Betiku  
*German Research Centre for Biotechnology,*  
*Biochemical Engineering Division,*  
*Mascheroder Weg 1, D-38124,*  
*Braunschweig, Germany*

Dr. Daniel Masiga  
*International Centre of Insect Physiology and Ecology,*  
*Nairobi,*  
*Kenya*

Dr. Essam A. Zaki  
*Genetic Engineering and Biotechnology Research Institute,*  
*GEBRI,*  
*Research Area,*  
*Borg El Arab, Post Code 21934, Alexandria Egypt*
Dr. Alfred Dixon  
*International Institute of Tropical Agriculture (IITA)*  
PMB 5320, Ibadan  
Oyo State, Nigeria

Dr. Sankale Shompole  
*Dept. of Microbiology, Molecular Biology and Biochemistry,*  
University of Idaho, Moscow,  
ID 83844, USA.

Dr. Mathew M. Abang  
*Germplasm Program*  
*International Center for Agricultural Research in the Dry Areas (ICARDA)*  
P.O. Box 5466, Aleppo, SYRIA.

Dr. Solomon Olawale Odemuyiwa  
*Pulmonary Research Group*  
*Department of Medicine*  
550 Heritage Medical Research Centre  
University of Alberta  
Edmonton  
Canada T6G 2S2

Prof. Anna-Maria Botha-Oberholster  
*Plant Molecular Genetics*  
*Department of Genetics*  
*Forestry and Agricultural Biotechnology Institute*  
*Faculty of Agricultural and Natural Sciences*  
University of Pretoria  
ZA-0002 Pretoria, South Africa

Dr. O. U. Ezeronye  
*Department of Biological Science*  
Michael Okpara University of Agriculture  
Umudike, Abia State, Nigeria.

Dr. Joseph Hounhouigan  
*Maître de Conférence*  
*Sciences et technologies des aliments*  
*Faculté des Sciences Agronomiques*  
*Université d'Abomey-Calavi*  
01 BP 526 Cotonou  
République du Bénin

Prof. Christine Rey  
*Dept. of Molecular and Cell Biology,*  
*University of the Witwatersand,*  
*Private Bag 3, WITS 2050, Johannesburg, South Africa*

Dr. Kamel Ahmed Abd-Elsalam  
*Molecular Markers Lab. (MML)*  
*Plant Pathology Research Institute (PPathRI)*  
*Agricultural Research Center, 9-Gamma St., Orman,*  
12619,  
Giza, Egypt

Dr. Jackie Hughes  
*Research-for-Development*  
*International Institute of Tropical Agriculture (IITA)*  
*Ibadan, Nigeria*

Prof. Greg Blatch  
*Head of Biochemistry & Senior Wellcome Trust Fellow*  
*Department of Biochemistry, Microbiology & Biotechnology*  
*Rhodes University*  
*Grahamstown 6140*  
*South Africa*

Dr. Beatrice Kilel  
P.O Box 1413  
Manassas, VA 20108  
USA

Dr. Robert L. Brown  
*Southern Regional Research Center,*  
*U.S. Department of Agriculture,*  
*Agricultural Research Service,*  
*New Orleans, LA 70179.*

Dr. Deborah Rayfield  
*Physiology and Anatomy*  
*Bowie State University*  
*Department of Natural Sciences*  
*Crawford Building, Room 003C*  
*Bowie MD 20715, USA*
Dr. Marlene Shehata  
*University of Ottawa Heart Institute*  
Genetics of Cardiovascular Diseases  
40 Ruskin Street  
K1Y-4W7, Ottawa, ON, CANADA

Dr. Hany Sayed Hafez  
*The American University in Cairo,*  
Egypt

Dr. Clement O. Adebooye  
*Department of Plant Science*  
Obafemi Awolowo University, Ile-Ife  
Nigeria

Dr. Ali Demir Sezer  
*Marmara Üniversitesi Eczacilik Fakültesi,*  
Tibbiye cad. No: 49, 34668, Haydarpasa, Istanbul,  
Turkey

Dr. Ali Gazanchain  
P.O. Box: 91735-1148, Mashhad,  
Iran.

Dr. Anant B. Patel  
*Centre for Cellular and Molecular Biology*  
Uppal Road, Hyderabad 500007  
India

Prof. Arne Elofsson  
*Department of Biophysics and Biochemistry*  
Bioinformatics at Stockholm University,  
Sweden

Prof. Bahram Goliaei  
*Departments of Biophysics and Bioinformatics*  
Laboratory of Biophysics and Molecular Biology  
University of Tehran, Institute of Biochemistry  
and Biophysics  
Iran

Dr. Nora Babudri  
*Dipartimento di Biologia cellulare e ambientale*  
Università di Perugia  
Via Pascoli  
Italy

Dr. S. Adesola Ajayi  
*Seed Science Laboratory*  
Department of Plant Science  
Faculty of Agriculture  
Obafemi Awolowo University  
Ile-Ife 220005, Nigeria

Dr. Yee-Joo TAN  
*Department of Microbiology*  
Yong Loo Lin School of Medicine,  
National University Health System (NUHS),  
National University of Singapore  
MD4, 5 Science Drive 2,  
Singapore 117597  
Singapore

Prof. Hidetaka Hori  
*Laboratories of Food and Life Science,*  
Graduate School of Science and Technology,  
Niigata University,  
Niigata 950-2181,  
Japan

Prof. Thomas R. DeGregori  
*University of Houston,*  
Texas 77204 5019,  
USA

Dr. Wolfgang Ernst Bernhard Jelkmann  
*Medical Faculty, University of Lübeck,*  
Germany

Dr. Moktar Hamdi  
*Department of Biochemical Engineering,*  
Laboratory of Ecology and Microbial Technology  
National Institute of Applied Sciences and  
Technology.  
BP: 676. 1080,  
Tunisia

Dr. Salvador Ventura  
*Department de Bioquímica i Biologia Molecular*  
Institut de Biotecnología i de Biomedicina  
Universitat Autònoma de Barcelona  
Bellaterra-08193  
Spain

Dr. Claudio A. Hetz  
*Faculty of Medicine, University of Chile*  
Independencia 1027  
Santiago, Chile

Prof. Felix Dapare Dakora  
*Research Development and Technology Promotion*  
Cape Peninsula University of Technology,  
Room 2.8 Admin. Bldg. Keizersgracht, P.O. 652,  
Cape Town 8000,  
South Africa
Dr. Geremew Bultosa  
*Department of Food Science and Post harvest Technology*  
Haramaya University  
Personal Box 22, Haramaya University Campus  
Dire Dawa, Ethiopia

Dr. José Eduardo Garcia  
*Londrina State University*  
Brazil

Prof. Nirbhay Kumar  
*Malaria Research Institute*  
*Department of Molecular Microbiology and Immunology*  
*Johns Hopkins Bloomberg School of Public Health*  
E5144, 615 N. Wolfe Street  
Baltimore, MD 21205

Prof. M. A. Awal  
*Department of Anatomy and Histplogy,*  
Bangladesh Agricultural University,  
Mymensingh-2202, Bangladesh

Prof. Christian Zwieb  
*Department of Molecular Biology*  
*University of Texas Health Science Center at Tyler*  
11937 US Highway 271  
Tyler, Texas 75708-3154  
USA

Prof. Danilo López-Hernández  
*Istituto de Zoología Tropical,*  
*Facultad de Ciencias,*  
*Universidad Central de Venezuela.  
Institute of Research for the Development (IRD), Montpellier,*  
*France*

Prof. Donald Arthur Cowan  
*Department of Biotechnology,*  
*University of the Western Cape*  
Bellville 7535  
*Cape Town,*  
*South Africa*

Dr. Ekhaise Osaro Frederick  
*University Of Benin, Faculty of Life Science*  
*Department of Microbiology*  
P. M. B. 1154, Benin City, Edo State, Nigeria.

Dr. Luísa Maria de Sousa Mesquita Pereira  
*IPATIMUP R. Dr. Roberto Frias, s/n 4200-465 Porto*  
Portugal

Dr. Min Lin  
*Animal Diseases Research Institute*  
*Canadian Food Inspection Agency*  
Ottawa, Ontario,  
Canada K2H 8P9

Prof. Nobuyoshi Shimizu  
*Department of Molecular Biology,*  
*Center for Genomic Medicine*  
*Keio University School of Medicine,*  
*35 Shinanomachi, Shinjuku-ku*  
*Tokyo 160-8582,*  
*Japan*

Dr. Adewunmi Babatunde Idowu  
*Department of Biological Sciences*  
*University of Agriculture Abia*  
*Abia State,*  
*Nigeria*

Dr. Yifan Dai  
*Associate Director of Research*  
*Revivicor Inc.*  
*100 Technology Drive, Suite 414*  
*Pittsburgh, PA 15219*  
*USA*

Dr. Zhongming Zhao  
*Department of Psychiatry,*  
*PO Box 980126,*  
*Virginia Commonwealth University School of Medicine,*  
*Richmond, VA 23298-0126,*  
*USA*

Prof. Giuseppe Novelli  
*Human Genetics,*  
*Department of Biopathology,*  
*Tor Vergata University, Rome,*  
*Italy*

Dr. Moji Mohammadi  
*402-28 Upper Canada Drive*  
*Toronto, ON, M2P 1R9 (416) 512-7795*  
*Canada*
Prof. Jean-Marc Sabatier  
Directeur de Recherche Laboratoire ERT-62  
Ingénierie des Peptides à Visée Thérapeutique,  
Université de la Méditerranée-Ambrilia  
Biopharma inc.,  
Faculté de Médecine Nord, Bd Pierre Dramard,  
13916,  
Marseille cédex 20.  
France

Dr. Fabian Hoti  
PneumoCarr Project  
Department of Vaccines  
National Public Health Institute  
Finland

Prof. Irina-Draga Caruntu  
Department of Histology  
Gr. T. Popa University of Medicine and Pharmacy  
16, Universitatii Street, Iasi,  
Romania

Dr. Dieudonné Nwaga  
Soil Microbiology Laboratory,  
Biotechnology Center. PO Box 812,  
Plant Biology Department,  
University of Yaoundé I, Yaoundé,  
Cameroon

Dr. Gerardo Armando Aguado-Santacruz  
Biotechnology CINVESTAV-Unidad Irapuato  
Departamento Biotecnología  
Km 9.6 Libramiento norte Carretera Irapuato-León Irapuato,  
Guanajuato 36500  
Mexico

Dr. Abdolkaim H. Chehregani  
Department of Biology  
Faculty of Science  
Bu-Ali Sina University  
Hamedan,  
Iran

Dr. Abir Adel Saad  
Molecular oncology  
Department of Biotechnology  
Institute of graduate Studies and Research  
Alexandria University,  
Egypt

Dr. Azizul Baten  
Department of Statistics  
Shah Jalal University of Science and Technology  
Sylhet-3114,  
Bangladesh

Dr. Bayden R. Wood  
Australian Synchrotron Program  
Research Fellow and Monash Synchroscopy  
School of Chemistry Monash University Wellington Rd. Clayton,  
3800 Victoria,  
Australia

Dr. G. Reza Balali  
Molecular Mycology and Plant Pthology  
Department of Biology  
University of Isfahan  
Isfahan  
Iran

Dr. Beatrice Kilel  
P.O Box 1413  
Manassas, VA 20108  
USA

Prof. H. Sunny Sun  
Institute of Molecular Medicine  
National Cheng Kung University Medical College  
1 University road Tainan 70101,  
Taiwan

Prof. Ima Nirwana Soelaiman  
Department of Pharmacology  
Faculty of Medicine  
Universiti Kebangsaan Malaysia  
Jalan Raja Muda Abdul Aziz  
50300 Kuala Lumpur,  
Malaysia

Prof. Tunde Ogunsanwo  
Faculty of Science,  
Olabisi Onabanjo University,  
Ago-Iwoye.  
Nigeria

Dr. Evans C. Egwim  
Federal Polytechnic,  
Bida Science Laboratory Technology Department,  
PMB 55, Bida, Niger State,  
Nigeria
Prof. George N. Goulielmos  
*Medical School, University of Crete*  
Voutes, 715 00 Heraklion, Crete, Greece

Dr. Uttam Krishna  
*Cadila Pharmaceuticals limited*,  
India 1389, Tarsad Road,  
Dhalka, Dist: Ahmedabad, Gujarat, India

Prof. Mohamed Attia El-Tayeb Ibrahim  
*Botany Department, Faculty of Science at Qena*,  
South Valley University, Qena 83523, Egypt

Dr. Nelson K. Ojijo Olang’o  
*Department of Food Science & Technology*,  
JKUAT P. O. Box 62000, 00200, Nairobi, Kenya

Dr. Pablo Marco Veras Peixoto  
*University of New York NYU College of Dentistry*  
345 E. 24th Street, New York, NY 10010 USA

Prof. T E Cloete  
*University of Pretoria Department of Microbiology and Plant Pathology*,  
University of Pretoria, Pretoria, South Africa

Prof. Djamel Saidi  
*Laboratoire de Physiologie de la Nutrition et de Sécurité*  
Alimentaire Département de Biologie, Faculté des Sciences, Université d’Oran, 31000 - Algérie  
Algeria

Dr. Tomohide Uno  
*Department of Biofunctional chemistry, Faculty of Agriculture Nada-ku, Kobe*,  
Hyogo, 657-8501, Japan

Dr. Ulises Urzúa  
*Faculty of Medicine, University of Chile Independencia 1027, Santiago*,  
Chile

Dr. Aritua Valentine  
*National Agricultural Biotechnology Center, Kawanda*  
Agricultural Research Institute (KARI)  
P.O. Box, 7065, Kampala, Uganda

Prof. Yee-Joo Tan  
*Institute of Molecular and Cell Biology 61 Biopolis Drive*,  
Proteos, Singapore 138673  
Singapore

Prof. Viroj Wiwanitkit  
*Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok*  
Thailand

Dr. Thomas Silou  
*Universit of Brazzaville BP 389*  
Congo

Prof. Burtram Clinton Fielding  
*University of the Western Cape Western Cape, South Africa*

Dr. Brnčić (Brncic) Mladen  
*Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia.*

Dr. Meltem Sesli  
*College of Tobacco Expertise*,  
Turkish Republic, Celal Bayar University 45210, Akhisar, Manisa, Turkey.

Dr. Idress Hamad Attitalla  
*Omar El-Mukhtar University, Faculty of Science, Botany Department, El-Beida, Libya.*

Dr. Linga R. Gutha  
*Washington State University at Prosser, 24106 N Bunn Road, Prosser WA 99350-8694.*
Dr Helal Ragab Moussa  
*Bahray, Al-bagour, Menoufia, Egypt.*

Dr VIPUL GOHEL  
*DuPont Industrial Biosciences Danisco (India) Pvt Ltd 5th Floor, Block 4B, DLF Corporate Park DLF Phase III Gurgaon 122 002 Haryana (INDIA)*

Dr. Sang-Han Lee  
*Department of Food Science & Biotechnology, Kyungpook National University Daegu 702-701, Korea.*

Dr. Bhaskar Dutta  
*DoD Biotechnology High Performance Computing Software Applications Institute (BHSAI) U.S. Army Medical Research and Materiel Command 2405 Whittier Drive Frederick, MD 21702*

Dr. Muhammad Akram  
*Faculty of Eastern Medicine and Surgery, Hamdard Al-Majeed College of Eastern Medicine, Hamdard University, Karachi.*

Dr. M. Muruganandam  
*Department of Biotechnology St. Michael College of Engineering & Technology, Kalayarkoil, India.*

Dr. Gökhan Aydin  
*Suleyman Demirel University, Atabey Vocational School, Isparta-Türkiye,*

Dr. Rajib Roychowdhury  
*Centre for Biotechnology (CBT), Visva Bharati, West-Bengal, India.*

Dr Takuji Ohyama  
*Faculty of Agriculture, Niigata University*

Dr Mehdi Vasfi Marandi  
*University of Tehran*

Dr Fügen DURLU-ÖZKAYA  
*Gazi University, Tourism Faculty, Dept. of Gastronomy and Culinary Art*

Dr. Reza Yari  
*Islamic Azad University, Boroujerd Branch*

Dr Zahra Tahmasebi Fard  
*Roudehen branche, Islamic Azad University*

Dr Albert Magri  
*Giro Technological Centre*

Dr Ping ZHENG  
*Zhejiang University, Hangzhou, China*

Dr. Kgomotso P. Sibeko  
*University of Pretoria*

Dr Greg Spear  
*Rush University Medical Center*

Prof. Pilar Morata  
*University of Malaga*

Dr Jian Wu  
*Harbin medical university, China*

Dr Hsiu-Chi Cheng  
*National Cheng Kung University and Hospital.*

Prof. Pavel Kalac  
*University of South Bohemia, Czech Republic*

Dr Kürsat Korkmaz  
*Ordu University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition*

Dr. Shuyang Yu  
*Department of Microbiology, University of Iowa Address: 51 newton road, 3-730B BSB bldg. Iowa City, IA, 52246, USA*

Dr. Binxing Li
Dr. Mousavi Khaneghah  
*College of Applied Science and Technology- Applied Food Science, Tehran, Iran.*

Dr. Qing Zhou  
*Department of Biochemistry and Molecular Biology, Oregon Health and Sciences University Portland.*

Dr Legesse Adane Bahiru  
*Department of Chemistry, Jimma University, Ethiopia.*

Dr James John  
*School Of Life Sciences, Pondicherry University, Kalapet, Pondicherry*
Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The cover letter should include the corresponding author’s full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author’s surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process
All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review. Decisions will be made as rapidly as possible, and the journal strives to return reviewers’ comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AJFS to publish manuscripts within weeks after submission.

Regular articles
All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors’ full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited. Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard Abbreviations should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer’s name and address. Subheadings should be used. Methods in general use need not be described in detail.
Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by 'et al'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works.

Examples:
Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Short Communications
Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.
**Fees and Charges:** Authors are required to pay a $650 handling fee. Publication of an article in the African Journal of Biotechnology is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

**Copyright: © 2014, Academic Journals.**

All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

**(Disclaimer of Warranties)**

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the AJB, whether or not advised of the possibility of damage, and on any theory of liability.

This publication is provided “as is” without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.
Table of Contents: Volume 13 Number 50, 10 December, 2014

ARTICLES

Antenna Elicitation And Behavioral Responses Of Oriental Fruit Moth, Grapholita Molesta, To Allyl Cinnamate
M. Giner, M. Balcells and J. Avilla

Phytochemical, Proximate And Anti-Nutrient Compositions Of Four Leafy Vegetables Used In South Eastern Nigeria
Abu Ngozi E., Ozoagudike Chinenye M. and Akaneme Florence I.

Physicochemical, Organoleptic And Nutritional Characteristics Of Four Sweet Cassava (Manihot Op) Varieties
E. Njoh Ellong, C. Billard and S. Adenet

Seed Pre-Treatment Methods For Improving Germination Of Acacia Tortilis
So Hanaoka, Norio Nakawa, Norihisa Okubo, Stephen Fredrick Omondi and Jason Kariuki

Chrysodeixis Includens (Lepidoptera: Noctuidae) On Soybean Treated With Resistance Inducers
Paulo Vinicius de Souza, Bruna Ribeiro Machado, Marcelo Mueller de Freitas, Fernanda Correa, André Cirilo de Sousa Almeida and Flávio Gonçalves de Jesus

Novel Carrier System For Enhancing Oral Delivery Of Metformin
Adedokun, M. O., Momoh, M. A., Kenechukwu, F. C., Umeh, O. N. C., Okpalanedu, C. M. J. and Ofokansi, K. C.

Differential Responses Of Growth, Chlorophyll Content, Lipid Peroxidation And Accumulation Of Compatible Solutes To Salt Stress In Peanut (Arachis Hypogaea L.) Cultivars
Meguekam Liliane Tekam, Taffouo Victor Désiré, Grigore Marius-Nicusor, Zamfirache Magdalena Maria, Youmbi Emmanuel and Amougou Akoa

Antidiabetic Activity And Acute Toxicity Evaluation Of Aqueous Leaf Extract Of Vernonia Amygdalina
M. A. Momoh, M. O. Adedokun, A. T. Mora and A. A. Agboke
Female sex pheromones have been used in pest control since the 90s; attracting males to baited traps (mass-trapping and monitoring) or avoiding (or reducing) mating in fields under mating disruption. By contrast, little is done among the use of male sex pheromones in pest control. Allyl cinnamate was evaluated as potential oriental fruit moth (Grapholita molesta, Busck) (Lepidoptera: Tortricidae) behaviour modifier, after recording positive electroantennographical responses in both male and female moths. Females over-responded in front of sources of allyl cinnamate at short distances, and both male and female showed typical pre-mating behavioural responses at mid-distances (in a wind tunnel). Males responded showing its hair-pencils and wing fanning and females started with wing fanning, curling abdomen and locating position opposite the source of allyl cinnamate. The same effect was observed in front of trans-ethylcinnamate, the main component of G. molesta male sex pheromone. Results here indicate a putative role of male sex pheromones (or chemically related compounds as allyl cinnamate) in oriental fruit moth integrated pest control. Understanding the role of male sex pheromones and chemically related compounds could help in the development of new pest control strategies.

Key words: Electroantennography (EAG), male sex pheromone, allyl ester, trans-ethyl cinnamate, wind-tunnel, behaviour modifier.

INTRODUCTION

Grapholita molesta (Busck) (Lepidoptera: Tortricidae), the oriental fruit moth, is a key-pest in most stone-fruit productive areas worldwide (Rotschild and Vickers, 1991), and it is also known to cause damages in apples at the end of the season (Kovanci et al., 2004). They firstly feed shoots, and after in fruits, causing damage on young trees and deprecating economic value of fruits (González, 2003). Moreover, feeding wounds are easily infected by brown rot (Garcia et al., 2004; Holb, 2004), increasing fruit depreciation and reducing its marketability.

Chemical control is still being the most used technique to maintain G. molesta populations under economic
thresholds, but mating disruption based on use of female sex pheromones, are being greatly spread as a way to avoid insecticide negative effects (L'chev et al., 2004; Devine and Furlong, 2007; Kong et al., 2014).

Female sex pheromones are used to monitor pest populations aimed to do chemical treatments in the best moment, to bait traps in mass-trapping and also for attract-and-kill strategies (Damos et al., 2014). In all cases, females are not the target, although some authors have reported an effect on female behaviour by exposure to their own sex pheromone (Stelinski et al., 2006; Gökçe et al., 2007; Kuhns et al., 2012). To increase the attractive effect on females, some volatile compounds were successfully added to pheromonal blends (Natale et al., 2003, 2004; Piñero and Dorn, 2007).

By contrast, few Lepidopteran male sex pheromones were studied even playing an important role in mate acceptance (Landolt and Heath, 1989; Hillier and Vickers, 2011). In the case of oriental fruit moth, a male sex pheromone is described; trans-ethyl cinnamate which is the main component. It is emitted by *G. molesta* males on their hair-pencils as aphrodisiac (Birch and Hefetz, 1987), increasing mating success at short distances (Baker et al., 1981; Löfstedt et al., 1990).

The study of courtship behaviour and mate acceptance could help to improve pest control based in the use of synthetic pheromones. A highly stereotypic sequence of behaviours are described to occur before mating (Curkovic et al., 2006), so the interference in this sequence could reduce mating and, consequently, pest populations.

Allyl cinnamate, chemically related with oriental fruit moth male main component of pheromone (Figure 1), have been described as female attractant on other two Tortricidae pests and so, an effect in oriental fruit moth was be expected.

This work describes for the first time allyl cinnamate as antenna elicitor and behaviour modifier of *G. molesta* moths, and contributes to the knowledge of oriental fruit moth courtship behaviour aim to be used into an integrated pest management (IPM) program. Differential effect than observed in other Tortricidae species is suggested due to similarities of allyl cinnamate with the main component of *G. molesta* male pheromone.

**MATERIALS AND METHODS**

**Insects**

*G. molesta* laboratory strain established in IRTA Research Centre (Spain) in 2005 from an established colony from field-collected individuals (Dr. F. Molinari, Piacenza, Italy) was used in all bioassays. Larvae were reared on semi-artificial diet (Ivaldi-Sender 1974) under long photoperiod (16L: 8D) and 24±1°C. Pupae were sorted by sex and placed into plastic cages (d= 15 cm, H= 5 cm) in a separated chamber at 24±1°C under the same photoperiod to obtained virgin adults. To obtain mated female individuals for EAG recordings and wind-tunnel behaviour assay, 2-3 couples of pupae were kept in the same cage.

Moths were used the second or third day after emergence and in behavioral assays 3 h before light off [peak of responsiveness of males to female sex pheromone and female gland extrusion (Baker and Cardé, 1979)].

The mating status of females was ascertained by the presence of spermatophore, which was determined after EAG recording or tunnel bioassay. Males maintained in the same case than females that were ascertained to be mated were differentially assessed than males that were kept in cages with no presence of females.

**Chemicals**

Allyl cinnamate and trans-ethyl cinnamate used in all bioassays were purchased by Sigma-Aldrich (www.sigmaaldrich.com). The main compound of *G. molesta* female sex pheromone was purchased by Pherobank (www.pherobank.com). All compounds had a minimum of 98% purity.

**Electrophysiological assays**

An EAG apparatus from Syntech (www.syntech.nl) was used. Signals after apparatus application (mV) were amplified (100×) and filtered (DC to KHz) with an ID-2 interface (Syntech), digitized on a PC and analysed with the EAG2000 program.

Antenna was carefully cut from an insect that was previously anesthetized with ice and then immobilized using a fine needle. Another cut was done at the end of the antenna using a scalpel. Then, antenna was placed between EAG electrodes, using electrode gel (www.parkerlabs.com/signagel.asp) to facilitate connexion between antenna and electrodes. Each stimulus was presented by first applying 0.1 µg to a piece of filter paper (2 × 2 cm). The piece of paper was then reinserted into a Pasteur pipette, which was placed so that the tip of the pipette was 5 cm from the antenna. A puff of air (300 mL min⁻¹) through the pipette then carried the stimuli to the antenna.

At least 10 antennae per sex and mating status were used in the bioassay. Five consecutive puffs (separated by 30 s) of the allyl cinnamate and control puffs with only solvent (acetone) were applied to each antenna in randomized order. No fatigue was observed in any antennae used in the bioassay.

For each antenna, the response to allyl cinnamate was calculated as the mean response to five puffs each, and was corrected by the mean of corresponding acetone puffs (EAG corrected = mean EAG compound – mean EAG control). Mean corrected EAG response of allyl cinnamate for each sex and state of mating were transformed [log (x+1)] to normalize the data, and then compared by one-way ANOVA, followed by Tukey-Kramer HSD test (P < 0.05) using the JMP 8.0.1 program (www.jmp.com).

**Behavioural assays**

First assay was done to assess virgin female responses to allyl cinnamate at short distances. 0.1 micrograms of allyl cinnamate dissolved in acetone were applied in a filter paper (2 x 2 cm) and then, the filter paper was introduced in a Petri dish (10 cm diameter, 5 cm height) containing female moths (n= 10, three repetitions). Female behavioural responses were recorded during 10 min, and insects were maintained in Petri-dishes during 24 h. Viability of insects at the end of the assay was recorded. The same, but applying only acetone in the filter paper, was done as control (n= 10, three repetitions). Percent of insects showing pre-mating behaviour against allyl cinnamate or control, and mortality among treatment and control were compared by X² test (P < 0.05) using the GraphPad program (www.graphpad.com).

Second assay aim to assess the effect of allyl cinnamate at mid-
distances was done in a wind tunnel (50 cm high, 200 cm long and 50 cm wide) situated in a room maintained at 23±2°C. Light was supplied by a fluorescent light situated on the ceiling of the room (200 lux) and two ventilators either on the side of the wind tunnel operated simultaneously, produced an air movement of 0.15 cm s⁻¹ through the tunnel. At least 20 mated and 20 virgin female moths, and 20 naïve and 20 no-naïve male moths were observed in front of each stimulus for 3 min. Each insect was assessed individually and only once. Red rubber septa of 8 mm (Sigma-Aldrich, Spain) situated 150 cm from the insect starting point and using a metal stand, held at a height of 20 cm, were used to place source, baited with 10 µg of stimulus. Septa with solvent alone (10 µL acetone) were also assessed, as control. Percent of insect showing a specific behaviour for each group and stimulus was compared by \( \chi^2 \) test using a contingency table \( (P < 0.05) \) with the GraphPad program.

The wind tunnel was cleaned with acetone after each experimental day and used material was washed with acetone and oven-dried at 200°C overnight.

**RESULTS**

Allyl cinnamate elicited antenna of both male and female *G. molesta*. No differences in antenna elicitation among mated and un-mated female moths were recorded \( (0.788\pm 0.289 \text{ mV} \) and \( 0.822 \pm 0.379 \text{ mV}, \) respectively \( (P > 0.05)) \). Neither among naïve and no-naïve males \( (1.037 \pm 0.308 \text{ mV} \) and \( 1.429 \pm 0.289 \text{ mV}, \) respectively \( (P > 0.05)). \)

When *G. molesta* female moths were introduced into Petri-dishes containing allyl cinnamate, typical behaviour preceding mating was triggered \( \text{insect movement, walking toward, hair-pencil extrusion and retraction, wing fanning, wing vibrating, flight and quick wing movement, cleaning antenna with legs, abdomen movement} \) (Baker, 1989)).

Notable increase of wind fanning was observed in all individuals (in most cases, moth remain winging on the Petri-dish floor moving the abdomen) \( \chi^2 = 196, \) df = 1, \( P < 0.001 \). The increase of moth activity conducted up to moth exhaustion; reflected by mortality observed in front of allyl cinnamate \( (100\%) \) compared to controls \( (< 10 \%) \) \( \chi^2 = 185, \) df = 1, \( P < 0.001 \).

In wind-tunnel, more female moths showed the behaviour “clean antenna” in the presence of allyl cinnamate and ethyl cinnamate than in front of control sources. No oriented flight was recorded in front of control sources and significant lower number of females showed mating behaviour compared to sources baited with allyl cinnamate or ethyl cinnamate. If we compare the responses of female moth that start flight, no significant differences were observed among control, ethyl cinnamate and the lowest dose of allyl cinnamate tested. By contrast, a significant higher percent of females started flight in front of sources baited with 50 µg of allyl cinnamate, and most of them showed an oriented flight to the source, even no contacts were recorded (Figure 2).

All males cleaned its antenna, displayed typical behaviour that precedes mating and showed its hair-pencils in front of allyl cinnamate and the main component of sex (both male and female sex pheromone) in wind-tunnel, while not in front of control sources. Male fluttering was only observed in the presence of female pheromone and allyl cinnamate, and mostly started flight in the presence of female sex pheromone and allyl cinnamate. Contacts were only recorded in front of main component of female sex pheromone (Figure 3).

**DISCUSSION**

To our knowledge, this is the first report describing *G. molesta* antenna elicitation by allyl cinnamate. Antenna elicitation by allyl cinnamate was independent of the state of mating, as previously observed in *C. pomonella* and *L. botrana* (not published data). *G. molesta* antenna elicitation was in the same range than recorded for the main components of sex pheromones giving an idea of the high affinity of allyl cinnamate to *G. molesta* antennae receptors.

Due to similarities in the chemical structure of allyl cinnamate and trans-ethyl cinnamate (main component of *G. molesta* male sex pheromone) an effect in mating process is suspected. Moths perceived allyl cinnamate, showing “cleaning antennae” behaviour, confirming results from EAG recordings, and all typical behaviours preceding mating were observed in presence of allyl cinnamate [insect movement, walking toward, hair-pencil extrusion and retraction, wing fanning, wing vibrating, flight and quick wing movement, cleaning antenna with legs, abdomen movement, female touch male abdomen, end-to-end position, wing directed to floor, oviposition move-ment] (Baker, 1989)). The energy needed to maintained these movements during long time \( (24 \text{ h presence of allyl cinnamate}) \) drives to the insect death due to exhaustion. This fact could be useful in pest control; all energy overused when allyl cinnamate is detected reduces time and energy that moth could actually use to find a mate, copulate, find a place to oviposit and oviposit. If one of these series is disrupted, a negative effect in the next generation (population reduction) is expected.

Ethyl cinnamate acts as aphrodisiac compound on females and it seems that males are attracted to ethyl cinnamate with the aim to ‘sneak’ copulations with females that are actively being courted by other males (Baker and Cardé, 1979). Allyl cinnamate effect could also be related with mating success, as a difference than ethyl-cinnamate - which acts at short distances (Baker et al., 1981); it seems that could be detected and conducted toward longer distances. Assays aim to discern the effect of both components at different ratios (female sex pheromone and allyl cinnamate) when applied together, and in field assays are necessary to ascertain this pest control proposal. If allyl cinnamate was joined to conventional mating disruption, an effect in female and male behaviour has to be expected; not attraction would be produced (as a difference than female sex pheromone), but a display of pre-mating behaviours. The time and energy spent on pre-mating behaviour could reduce the number of effective mating and so, population in next generations.
Figure 1. Chemical structures of allyl cinnamate and ethyl cinnamate.

Figure 2. Percent of *G. molesta* virgin females showing a specific behaviour in wind-tunnel assay in front of septum containing 10 µg of allyl cinnamate, 10 µg ethyl cinnamate, 100 µg of allyl cinnamate or solvent during 3 min recording. cin = Allyl cinnamate; Columns within a specific behaviour followed by the same letter are not significantly different. $\chi^2$ test shows homogeneity, (2 x 2) $P < 0.05$.

Figure 3. Percent of *G. molesta* virgin males showing a specific behaviour in wind-tunnel assay in front of septum containing 10 µg of main component of female sex pheromone, 10 µg of allyl cinnamate, 10 µg of ethyl cinnamate, or solvent during 3 min recording.
Additionally, allyl cinnamate is used as aroma (http://eur-lex.europa.eu/) so low toxic effects in not-target organisms are suspected and it could be synthesized from glycerol (Escribà et al., 2009, 2011), so its use could help to find an alternative to glycerol surplus (www.biodiesel.org).

**Conflict of Interests**

The author(s) have not declared any conflict of interests.

**ACKNOWLEDGMENTS**

M. G. was financed by fellowship nº BES-2008-004779. M. G., M. B. and J. A. were supported by Spanish Ministry of Economy and Competitiveness research grant AGL 2010-17486. Authors are grateful to C. Gemeno (University of Lleida) by insect supply and wind-tunnel equipment supervision.

**REFERENCES**


González RH (2013) Las polillas de la fruta en Chile (Lepidoptera: Tortricidae; Pyralidae). Universidad de Chile, serie de ciencias agronómicas 9. Santiago de Chile, Chile. 188pp.


Phytochemical, proximate and anti-nutrient compositions of four leafy vegetables used in South Eastern Nigeria

Abu Ngozi E.*, Ozoagudike Chinenyen M. and Akaneme Florence I.

Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Received 2 October, 2014; Accepted 24 November, 2014

Chemical constituents of plants are influenced by environmental factors and fluctuations just as many other polygenic traits. Four different green leafy vegetables commonly used in the diets of South Eastern Nigeria were analyzed with a view to determine the phytochemicals, proximate and anti-nutrient compositions of these ecotypes. The vegetables, of which three are spices, are scent leaf (*Ocimum gratissimum* L.), fluted pumpkin (*Telfaria occidentalis*), amaranth globe (*Gongronema latifolium* Benth.) and ashanti pepper (*Piper guineense* Schumach. and Thonn.) leaves. The preliminary phytochemical analysis indicates the presence of the phytochemicals from trace amounts to strongly present. The quantitative tests showed that *O. gratissimum* had significantly (P ≤ 0.05) the highest tannin content of 1074.94±0.009 mg/100 g. Significant variations were observed in all the other phytochemicals except in alkaloid and phenol content. Proximate and antinutrient compositions showed significant variation in the different vegetables. *T. occidentalis* had highest values in ash (13.51%), crude fibre (33.52%), protein (25.49%) and phytate (8.58 mg/100 g) contents. The results obtained in this study clearly indicate that the four leafy vegetables are readily available sources of nutrients and prove the extensive use of these vegetables in ethnomedicine; and their potential in drug formulation.

Key words: Alkaloid, ethnomedicine, proximate, spice, vegetables.

INTRODUCTION

Vegetables are the edible parts of herbaceous plants that are consumed wholly or in parts, raw or cooked as part of main dish or salad; they may be aromatic, bitter or tasteless (Dhellot et al., 2006). They include leaves, stems, roots, flowers, seeds, fruits, and bulbs. Leafy vegetables however, are source of macro and micro-nutrients that play major role in maintaining healthy living. They are regular ingredients in the diet of the average Nigerian and provide appreciable amounts of nutritive minerals (Ajewole, 1999). Even though the bulk of their weight is water, leafy vegetables represent a veritable natural pharmacy of minerals, vitamins and phytochemicals (George, 2003). Leafy vegetables investigated in this experiment were *Ocimum gratissimum*, *Piper guineense*, *Gongronema latifolium* and *Telfaria occidentalis*.
T. occidentalis commonly called fluted pumpkin is a tropical vine grown in West Africa as a leafy vegetable. They belong to the family Cucurbitaceae (Akoroda, 1990). The fluted gourd grows in many nations of West Africa but is mainly cultivated in Nigeria, used primarily in soups and herbal medicines (Akoroda, 1990). Fluted pumpkin leaves have a lot of nutritive value. This made the leaves and tender stems potentially useful as food supplements (Nkang et al., 2002). The leaves contain a high amount of vitamins A and C, antioxidants, hepatoprotective and antimicrobial properties (Oboh et al., 2006). The leaf extract is useful in the management of cholesterolemia, liver problems and impaired defense immune systems (Esseyin et al., 2005). The leaves are rich in iron and play a key role in the cure of anaemia; they are also noted for lactating properties and are in high demand for nursing mothers (Okoli and Mgbeogwu, 1983). In Nigeria the fresh leaves are ground and the juice used as tonic by women that have just given birth; its high iron content assists in the replenishment of lost blood, used for treatment of anaemia, chronic fatigue and diabetes (Aderibigbe et al., 1999; Dina et al., 2006; Alada, 2010). Longe et al. (1983) reported that minerals such as calcium, potassium, magnesium, iron, sodium and phosphorus, antioxidants and vitamins such as thiamine, riboflavin, nicotinamide and ascorbic acid were present in fluted pumpkin. Phytochemicals such as phenols were concentrated in the testa, pulp and husk and amino acids such as alanine, aspartate, glycine, leucine and many others (Fasuyi and Nonyerem, 2007).

P. guineense (also called African pepper, Ashanti pepper, Benin pepper, false cube, Guinea cube, uziza pepper and Guinea pepper) belonging to the family Piperaceae are semi-cultivated in countries such as Nigeria where the leaves and seeds are used as flavouring agent (Asawalam et al., 2007). Studies have shown that apart from the use of these plants as spices and condiments, they have several other wide applications in the local treatment and management of many diseases. It provides oil used as aromatic in the drink industry and also medicinally (Sumathykutty et al., 1999). Okwu (2001) reported that phytochemical analyses of P. guineense showed the presence of alkaloids, flavonoids, saponins, tannins, resins and essential oils. These phytochemicals have a lot of pharmacological properties as proved by earlier studies (Okwu, 2001). He also reported that the presence of alkaloids signified the possession of medicinal properties within the leaves. The flavonoids possessed antioxidant activity, anti-inflammatory and antiviral activities (Okwu, 2001).

O. gratissimum is a herbaceous plant which belongs to the family Lamiaceae (Calixto, 2000). It is widely distributed in tropical and warm temperature regions (Okigbo and Ogbonnaya, 2006). O. gratissimum, also called scent leaf is grown in gardens and used as a tea leaf for fevers (Okigbo and Ogbonnaya, 2006). It is germicidal and has found wide use in toothpastes and mouth washes as well as topical ointments (Pessoa et al., 2003; Holets et al., 2003). It is used as an excellent gargle for some throats and tonsillitis. It is also used as an expectorant and a cough suppressant. The plant extract is used against gastrointestinal helminths of animals and man (Chitwood, 2003). Phytochemical studies show that both aqueous and methanolic extracts of O. gratissimum are rich in tannins, steroids, terpenoids, flavonoids and hydrogen-cyanide and equally has a good antioxidant activity (Afolabi et al., 2007).

G. latifolium (amaranth globe) belongs to the family Asclepiadaceae and is a tropical rainforest plant primarily used as spice and vegetable in food (Nwosu and Malize, 2006). It is listed among the twenty-eight medically important vegetables of South West Nigeria (Ayodele, 2008) and also as one of the aromatic plants of medicinal importance from Nigeria (Ogunwande et al., 2007). The leaves are sharp-bitter and sweet and widely used as a leafy vegetable and as a spice for sauces, soups and salads. G. latifolium is also widely used in West Africa for medicinal and nutritional purposes. Ugochukwu et al. (2003) reported that aqueous and Ethanolic extracts of Amaranth globe had hypoglycemic, hypolipidemic and antioxidative properties while Morebise et al. (2002) showed that it has anti-inflammatory properties. They also have industrial uses in brewing beer. Ogundipe et al. (2003) discovered that leaves of Amaranth globe were possible hop substitute for brewing beer and that water extracts of the powdered leaves gave low bitterness values, but extraction of the powdered leaves with organic solvents significantly increased analytical bitterness to levels comparable with hops.

It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect (Afolabi et al., 2007). The medicinal value and multiple biological functionalities of several plants are defined by their phytochemical constituents (Fallah et al., 2005). The medical values of leafy vegetables are dictated by their phytochemical and other chemical constituents (Fallah et al., 2005). However, these chemical constituents including the phytochemicals are influenced by environmental factors just as many other polygenic traits. Therefore, there is need to determine the chemical compositions of the eco-types grown at Enugu, South Eastern, Nigeria. The aim of this study was to determine the chemical compositions, proximate, mineral and phytochemical constituents, of four leafy vegetables, out of which three also serve as spices, and to relate the above to their nutritional and the ethno medicinal uses by the indigenous population of South Eastern Nigeria.

MATERIALS AND METHODS

Collection and identification of plant material

The leafy vegetables, O. gratissimum, P. guineense, G. latifolium and T. occidentalis, were collected from Enugu, in Enugu state, South Eastern Nigeria. The specimens were properly identified and
authenticined in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

**Preparation of plant extracts**

**Drying process**

The samples were thoroughly washed, air dried under shed for seven days at room temperature, to avoid loss of active compounds. The dried leaves were ground to powder using an electric grinder. The powdered samples were then stored in appropriately labeled air-tight bottles for further use (Trease and Evans, 2002).

**Extraction process**

Aqueous extracts were used for the phytochemical analysis. The process was hot water method following the procedure of Handa (2008) and Handa et al. (2008).

**Phytochemical, proximate and anti-nutrient analyses**

Both the proximate, antinutrients, qualitative and quantitative phytochemical analyses of the leafy samples of *O. gratissimum*, *P. guineense*, *G. latifolium* and *T. occidentalis* were carried out to detect the presence of some secondary metabolites using the standard methods by the Association of Official Analytical Chemistry (AOAC, 2002). The phytochemicals tested for were alkaloids, saponins, glycosides, tannins, steroid, terpenoid, flavonoids, Hydrogen cyanide and Phenols.

**RESULTS**

The qualitative analysis showed a trace amount of alkaloid in *T. occidentalis* and the presence of alkaloid in the other three samples. Glycoside was present in *O. gratissimum* and *T. occidentalis* but showed trace amount in *P. guineense* and *G. latifolium*. Saponin was in trace amount in all the four leaf samples. Phenol was present in *O. gratissimum*, strongly present in *P. guineense* and *G. latifolium* but very strongly present in *T. occidentalis*.

Similar variations were observed in the test for Tannin, hydrogen cyanide, terpenoid and steroid however, flavonoid was present in all the samples (Table 1). The quantitative assessment of the phytochemical constituents showed significant variations in the actual compositions of the different phytochemicals in the vegetables (Table 2). All the phytochemical components were significantly different from each other at P ≤ 0.05. The tannin content of the vegetables ranged from 716.6 mg/100 g in *P. guineense* to 1074.94 mg/100 g in *O. gratissimum*. The values for flavonoid, saponin, glycoside and steroid also ranged from 229.6 mg/100 g to 235.8 mg/100 g, 0.86 mg/g to 0.97 mg/g, 5.45 mg/g to 6.48 mg/g and 5.51 mg/g to 9.67 mg/g, respectively (Table 2). *G. latifolium* had the highest values in alkaloid and terpenoid while the least was recorded in *T. occidentalis*.

The result of the proximate composition on the leaves of *O. gratissimum*, *P. guineense*, *G. latifolium* and *T. occidentalis* showed that the moisture content ranged from 2.58% in *T. occidentalis* to 9.14% in *O. gratissimum* (Table 3). Percentage protein ranged from 17.16 to 25.49% among the vegetables. Variations in the percentage compositions of ash content, crude fibre, fat and carbohydrate were also observed in the different leafy vegetables. The Anti-nutrient composition showed the presence of Phytate and oxalate (Table 4). The value of phytate in *O. gratissimum* (1.26 mg/100 g) differed significantly from *P. guineense* (1.57 mg/100 g) but there was no significant difference between the values in *T. occidentalis* and *G. latifolium*. The values for oxalate across the vegetables ranged from 3.89 - 8.58%.

**DISCUSSION**

The study revealed that the extracts of these vegetables are rich in phytochemicals such as saponins, alkaloids, flavonoids, terpenoids, steroids, glycosides, tannins, hydrogen cyanide and phenols. Most of these
Table 2. Quantitative phytochemical compositions of O. gratissimum, T. occidentalis, G. latifolium and P. guineense leaves.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tannin (mg/100 g)</th>
<th>Flavonoid (mg/100 g)</th>
<th>Glycoside (mg/g)</th>
<th>Saponin (mg/g)</th>
<th>Steroid (mg/g)</th>
<th>Terpenoid (mg/100 g)</th>
<th>Phenol (mg/100 g)</th>
<th>HNC (mg/g)</th>
<th>Alkaloid (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. gratissimum</td>
<td>1074.94±0.009*</td>
<td>2320.09±0.008</td>
<td>6.39±0.004</td>
<td>.91±0.006</td>
<td>7.19±0.004</td>
<td>11.78±0.006</td>
<td>983.88±0.006</td>
<td>.65±0.005</td>
<td>1406.51±0.006</td>
</tr>
<tr>
<td>T. occidentalis</td>
<td>859.96±0.008</td>
<td>233.34±0.006</td>
<td>6.4±0.006</td>
<td>.89±0.004</td>
<td>5.51±0.003</td>
<td>10.64±0.005</td>
<td>1403.23±0.005</td>
<td>.38±0.002</td>
<td>677.51±0.005</td>
</tr>
<tr>
<td>G. latifolium</td>
<td>906.91±0.006</td>
<td>229.63±0.004</td>
<td>5.84±0.004</td>
<td>.66±0.006</td>
<td>5.67±0.005</td>
<td>12.37±0.004</td>
<td>1291.26±0.005</td>
<td>.45±0.005</td>
<td>1577.24±0.005</td>
</tr>
<tr>
<td>P. guineense</td>
<td>716.63±0.006</td>
<td>235.81±0.008</td>
<td>5.45±0.007</td>
<td>.97±0.009</td>
<td>5.62±0.004</td>
<td>11.32±0.005</td>
<td>1290.33±0.006</td>
<td>.65±0.006</td>
<td>1132.79±0.006</td>
</tr>
<tr>
<td>L.S.D (P ≤ 0.05)</td>
<td>0.012</td>
<td>0.009</td>
<td>0.007</td>
<td>0.007</td>
<td>0.009</td>
<td>NS</td>
<td>0.008</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

HNC, Hydrogen cyanide. * Values are mean ± SD; NS, not significant. ** Values followed by different letters are significantly different from each other.

Table 3. Proximate Composition (%) of the leaves of O. gratissimum, P. guineense, G. latifolium and T. occidentalis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture</th>
<th>Protein</th>
<th>Ash</th>
<th>Fat</th>
<th>Fibre</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. gratissimum</td>
<td>9.14±0.003</td>
<td>19.88±0.004</td>
<td>10.58±0.007</td>
<td>10.25±0.006</td>
<td>12.57±0.006</td>
<td>37.58±0.018</td>
</tr>
<tr>
<td>T. occidentalis</td>
<td>2.56±0.003</td>
<td>25.49±0.004</td>
<td>13.52±0.005</td>
<td>12.62±0.004</td>
<td>33.52±0.008</td>
<td>12.28±0.020</td>
</tr>
<tr>
<td>G. latifolium</td>
<td>4.63±0.005</td>
<td>17.16±0.003</td>
<td>10.13±0.003</td>
<td>10.14±0.004</td>
<td>16.58±0.005</td>
<td>41.36±0.007</td>
</tr>
<tr>
<td>P. guineense</td>
<td>4.63±0.004</td>
<td>19.36±0.007</td>
<td>11.39±0.004</td>
<td>14.63±0.004</td>
<td>21.37±0.004</td>
<td>28.62±0.013</td>
</tr>
<tr>
<td>L.S.D (P ≤ 0.05)</td>
<td>0.007</td>
<td>0.009</td>
<td>0.009</td>
<td>0.008</td>
<td>0.011</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Table 4. Anti-nutrient composition of O. gratissimum, T. occidentalis, P. guineense, and G. latifolium leaves.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Oxalate (mg/100 g)</th>
<th>Phytate (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. gratissimum</td>
<td>1.26±0.008</td>
<td>3.9±0.005</td>
</tr>
<tr>
<td>T. occidentalis</td>
<td>0.94±0.003</td>
<td>8.58±0.003</td>
</tr>
<tr>
<td>G. latifolium</td>
<td>0.94±0.006</td>
<td>7.8±0.002</td>
</tr>
<tr>
<td>P. guineense</td>
<td>1.57±0.003</td>
<td>7.8±0.007</td>
</tr>
<tr>
<td>L.S.D (P ≤ 0.05)</td>
<td>0.009</td>
<td>0.01</td>
</tr>
</tbody>
</table>

phytochemical constituents are potent bioactive compounds found in medicinal plant parts which are precursors for the synthesis of useful drugs. These phytochemicals generally have a wide range of pharmacological activities or actions (Louio et al., 2002). Alkaloids, the most revered of all phytochemicals are said to be pharmacologically active and their action are felt in the automatic nervous system, blood vessels, respiratory system and gastrointestinal tract (Louio et al, 2002). The observed presence and quantity of tannins in all the vegetables could be of great medicinal importance since tannins serve as good antioxidant (Okwu, 2001).

O. gratissimum had high values in all the phytochemicals tested in this study; this makes this vegetable and spice very important in the claimed pharmacological properties and uses in
ethnomedicine. Koleva et al. (2000) reported that the medicinal values of *O. gratissimum* lie in the phytochemical component which produces definite physiological actions. These components are associated with bioactivities having health impacts. From recent findings, *O. gratissimum* has proved to be useful in medication for people living with HIV and AIDS (Nwinyi et al., 2009). *P. guineense* extracts are, equally, good sources of antioxidants, which are widely believed to be important line defense against oxidative stress leading to a lot of diseases like insomnia, and diabetes.

Flavonoids were abundant in the four leafy vegetables but had significantly the highest value in *P. guineense*. They are recognised for their anti-oxidant, anti-carcinogenic, anti-microbial and anti-tumour properties (Del-Rio et al., 1997). Linus (1991) reported that flavonoids which are naturally occurring low molecular weight polyphenolic compounds located in fruits and vegetables are known to inhibit formation of plaques and streaks in arteries and as such hinder hypertension and other cardiovascular diseases. Del-Rio et al. (1997) noted that flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity. Phenols which are abundant in the four leafy vegetables have been reported to have potential as antioxidants due to their being effective hydrogen donors (Oboh and Akindahunsi, 2004) while Osuagwu and Nwosu (2006) noted that saponins were involved in ulcer protection and certain antimicrobial activity. Steroid having the highest value in *G. latifolium* was reported as being effective in regulating carbohydrate and protein metabolism and possesses anti-inflammatory properties (Omotayo and Borokini, 2012). In recent years, the prevention of cancer and cardiovascular diseases has been associated with the ingestion of spices and vegetables rich in natural antioxidants (Chu et al., 2002).

All the vegetables studied contain appreciable amount of protein which indicates that the vegetables can be used for building and repairing of body tissues, regulation of body processes and formation of enzymes and hormones. The fibre content was highest in *T. occidentalis*, followed by *P. guineense*, the substantial amount of fibre in all the vegetables showed that they can help in keeping the digestive system healthy and functioning properly. Fibre aids and speeds up the excretion of waste and toxins from the body, preventing them from sitting in the intestine or bowel for too long, which could cause a build-up and lead to several diseases (Hunt et al., 1980). The low percentage carbohydrate and fat contents in the vegetable could be an advantage in the diets of people based on age and body mass. Lipids among other things had been reported to help the human body to absorb fat-soluble vitamins such as vitamins A and E (Osborne and Voogt, 1978). That means that the low lipid content in these vegetables could be an advantage by helping uptake of water soluble vitamins. Low amounts of phytate and oxalate antinutrients were detected in the vegetables. The beneficial aspects of antinutrients and other bioactives have been discussed when used in low levels (Gemede and Ratta, 2014). In conclusion these vegetables contain low calories and negligible quantities of utilizable energy; hence, they are ideal for obese people who can satisfy their appetite without consuming much carbohydrate. All the plants studied have proved to be very important because of their chemical constituents, this explains their wide usage by the people of South Eastern Nigeria in diets and in ethnomedicine. Even though they have anti-nutrients, their values are too small to be harmful, therefore, consumption of these vegetables is encouraged both as food for nutrients and as medicine in ethnomedicine. The authors, therefore, suggest intensified research that would be directed towards harnessing the potentials of these vegetables in drug formulation and development in addition to their daily use in diets.

**Conflict of Interest**

The author(s) have not declared any conflict of interests.

**REFERENCES**


Physicochemical, organoleptic and nutritional characteristics of four sweet cassava (*Manihot opí*) varieties

E. Njoh Ellong, C. Billard and S. Adenet*

Pôle Agroalimentaire Régional de Martinique (P. A. R. M.) Habitation Petit Morne 97232 Le Lamentin, Martinique, France.

Received 5 November, 2013; Accepted 17 November, 2014

There exist a wide variety of tropical tubercles in Martinique French West Indies (F.W.I.). This study was carried out in order to investigate the development of local varieties of sweet cassava (*Manihot opí*). The purpose of this research was to enhance knowledge of the nutritional and agricultural characteristics of these tubers. The first step in the research project was to carry out a survey and screening of selected varieties, which was undertaken by the local department of agriculture throughout the region of Martinique. Phenotypic characterization and identification were then established. The four varieties were distinguished from each other by phenotypic, organoleptic, physicochemical and nutritional characteristics. Sweet cassava in Martinique are richer in nutritional compounds and vitamin C than ordinary potatoes. Their sensory specificity, high nutritional value and suitability for industrial processing have also been highlighted. Variety KM07 seemed to offer the best compromise between size, nutritional profile and sensory characteristics and would therefore be recommended for production.

Key words: Martinique, *Manihot opí*, physicochemical characteristics, nutritional characteristics, sensory profile, sweet cassava, gallic acid equivalent (GAE).

INTRODUCTION

Cassava belongs to the Euphorbiaceae family and can be divided into two categories: Arboaeae and Fructicoseae. Originally from South America, cassava was introduced into Martinique by the Caribbean Indians (Jackson et al., 1991; Silvestre and Arrauode, 1983). There are two varieties of cassava. *Manihot opí* is the sweet cassava, the roots and leaves of which are consumed fresh. *Manihot esculenta* Crantz (*Manihot utilissima*) is the bitter cassava and the most cultivated because its production yield is higher, despite being inedible without pre-treatment. It contains toxic cyanide glycosides, which, under the effect of an enzyme, are transformed into hydrogen cyanide (Nartey, 1968; Conn, 1969; McMahon et al., 1995). Hydrogen cyanide (HCN) is a strong inhibitor of many metallo-enzymes, especially cytochrome oxidase, a key enzyme in cellular respiration. Cyanogenic glycosides...
are synthesized by cassava from germination (Dunstan et al., 1996). There are high concentrations in parts of the plant that carry out photosynthetic activity and during the period when the plant grows the fastest (Louembe et al., 1997). Not all varieties of cassava contain the same amount of HCN, and predictions of cyanide contents exist (Sanchez et al., 2014). Sweet varieties contain 30 to 130 ppm of HCN when raw, bitter varieties 80 to 400 ppm, and very bitter varieties 275 to 500 ppm of HCN (Nassar and Ortiz, 2006). The global detoxification procedures for cassava have been reviewed by Coursey (1973), Lancaster et al. (1982) and Padmaja (1995). The effects of traditional as well as modified processing techniques in reducing the cyanogen levels have been studied by several scientists (Cooke, 1982; Fukuba et al., 1984; Nambisan and Sundaresan, 1985; Mahungu et al., 1987; O’Brien et al., 1992; Padmaja et al., 1994). The poisonous substance, present in larger quantities in the peel is removed by peeling, washing or cooking, sun drying or fermentation. Mild method for removal of cyanogens from cassava leaves with retention of vitamins and protein has been developed (Bradbury and Denton, 2014). The dried roots are processed into tapioca, cassava or flour. Tapioca is a feacula of cassava that is used to thicken soups, puddings, creams or custards. There are many cultivars of bitter varieties, for example, ‘Chasagana’, ‘Green cassava’, ‘Pilotin’, ‘Saint Vincent’, etc. (Germosén-Robineau, 1999).

Cassava is now widely grown in tropical countries. It grows best in wetland and sunny areas but accepts any type of soil (Jennings, 1995). It multiplies through cutting stems or tubers. Planting usually takes place during the rainy season. The harvest occurs 8 to 36 months later. Cassava is a shrub growing between 1 and 3 m high with tuberous roots and white flesh rich in starch. The skin of the bitter cassava tuber is thin and adherent, whereas in the sweet variety it is thick and easily removed. Its underground part comprises feeder roots which penetrate deep into the ground (up to 1 m) and tuberous roots located just below the soil’s surface. These roots form a bundle of 5 to 10 plants and can reach a length of 30 to 120 cm, with a diameter of 4 to 15 cm and a weight of 1 to 8 kg [it is these roots that are consumed (Cock, 1985)].

Nineteen million hectares of cassava are grown worldwide, including 12 million hectares in Africa, 2 million hectares in South America and 4 million hectares in Asia. World production reached 233 million tons in 2009 (FAOSTAT, 2009). Nigeria is the largest producer with 36 million tons, followed by Thailand (30 million tons) and Indonesia (22 million tons). In 2009, Martinique produced 194 tons of cassava, covering 76 ha. Cassava in Martinique is used mainly in the form of flour. The roots of sweet varieties are eaten boiled. In addition to the use of tubers and leaves for food, cassava is selected for starch production. The bitter cassava starch has a variety of uses and its properties are similar to those of potato starch (Delpeuch et al., 1978). In some countries, such as Brazil, a large-scale cassava industry has developed. The starch is used as a food thickener or as glue in stationery production. The majority of cassava imported to Europe is used for animal feed. Indeed, it constitutes a very good source of energy and the presence of fibres makes it very digestible. Asian countries such as Thailand and Cambodia specialize in its export.

In order to expand opportunities for consumption of cassava in the French West Indies (F.W.I.) the Trade Council of Guadeloupe initiated a study in 1997 in order to analyse the causes of the decline in production and consumption of cassava. This program also defined how to boost production. With the development of new eating habits, new ways to consume cassava have been identified, including in the form of bread (many traditional African methods lead to making cassava starch bread) and cassava couscous (also based on a method of making African "attiéké") (Regez and Mulumba, 1987). Other by-products were also valued, including glue made from starch and animal feed made from cassava leaves following detoxification. Investigation of ethanol productivity of cassava crop as a source of biofuel in tropical countries has been studied (Adelkekan, 2013).

In order to promote the production and consumption of cassava and integrate it into the daily diet, we decided to study the physicochemical and nutritional properties of four local varieties of cassava grown in Martinique. We wanted to compare the different qualities of these selected cultivars. This study is therefore an evaluation of cassava characteristics focussing on: (i) a sensorial analysis of four cassava cultivars and (ii) the physicochemical and nutritional characteristics of these four tubers.

MATERIALS AND METHODS

Plant material

Varieties were provided by the local department of agriculture and local producers. A minimum of 30 tubers were collected from each variety of cassava in order to have representative samples for each variety. A set of physical parameters was measured on each tuber (size and weight). They were then cleaned, peeled, sliced and steamed. Physicochemical analyses were carried out on cooked slices (texture and colour) or on crushed cooked pulp (energy, fibres, starch, polyphenols, sugars, minerals and vitamin C content).

Morphology and composition of sweet cassava

Morphological and physical characterization

The proximate analyses of cultivar size were carried out using a calliper for fruit length and width and a precision balance for cassava weight (Shimadzu UW4200HV). Texture parameters were measured with a LLOYD Instruments TA plus texture analyser, using a piston 4 mm in diameter and 30 mm/min speed. The tuber colours were determined with a Minolta CR-200 Chroma meter using the three parameters \( L^* \), \( a^* \) and \( b^* \) established by the International Commission on Illumination: \( L^* \) for lightness to

**Afr. J. Biotechnol.**
distinguish light colours from dark colours, $a'$ used to classify red to green colours, and $b'$ used to classify yellow to blue colours.

**Physicochemical characterization of the tuber**

For each analysis and sample, three replicates were performed.

**Dry matter content (DM):** Flesh samples (2 g of crushed, homogenized flesh) were collected from each cassava for DM determination in triplicate, using a ventilated oven at 70°C for 5 h at reduced pressure (-1 bar).

**Ash content:** Cassava ash content was calculated from a crushed sample (1 g) following heating to 525°C for 5 h as per the AOAC official method 923.03 (1996).

**Protein content:** The protein content was determined through the quantification of total nitrogen using Kjeldahl's method. Following mineralization of the sample (2 g) in 25 ml of 95% H2SO4 using a catalyst and a Turbotherm mineralizer (Gerhardt Laboratory Systems, Königswinter, Germany), the mineralize was distilled using soda in a Vapodest distiller (Gerhardt Laboratory Systems, Königswinter, Germany). The distillate was collected in 40 ml of boric acid 40 g.l⁻¹ with the use of some drops of Tashiro's indicator. The distillate was titrated by hydrochloric acid HCl (0.1 N).

**Lipid content:** Lipids were extracted from the sample (2 g) after acid hydrolysis with 50 ml of 8 N HCl, at 80°C. The mixture was filtered and rinsed with boiling water until a neutral pH was obtained, after which the filters were dried. The residues were placed in glass cartridges with 140 ml of petroleum benzene and some pumice stones to extract the lipids in a Soxhlet extractor (Gerhardt Laboratory Systems, Königswinter, Germany). After extraction, the cartridges were oven dried at 101°C.

**Carbohydrate content:** This was obtained by finding the difference [(dry extract – (ash + lipids + proteins)]. Carbohydrates represent the total fibre, starch and sugar content.

**Energy value:** Energy value was determined by adding lipid, carbohydrate and protein contents.

**Starch content:** The starch content was measured using the K-TSTA 11/05 Megazyme enzymatic kit (Megazyme, Wicklow, Ireland). The samples were crushed cooked flesh. The absorbance at 334 nm, proportional to the amount of glucose released by the hydrolysis of starch, was read using a spectrophotometer (JENWAY 7305).

**Fibre content:** Fibre content was determined with the AOAC 985.29 method.

**Polyphenol content:** Total polyphenols were determined using Folin and Ciocalteu's method described in Georgé et al. (2005). Only raw extracts were analysed.

**Ca, K, P, Zn and Mg:** Ca, K, P, Zn and Mg contents were determined with the Cofrac program 60. Ca, K, P, Zn and Mg rates were determined by atomic absorption spectrometry, respectively at 422.7, 766.5, 430, 213.8 and 285.2 nm.

**pH:** About 25 g of flesh (cooked or raw) was blended with ~ 250 ml deionised water (10% w/w) for 30 min using a magnetic stirrer. The pH of the blended solution was determined at ambient temperature with a Sentix 81 (WTW) probe.

**Enzymology:** Measures were carried out on raw and cooked cassava. 2 g of samples were dissolved in 0.5 mM ascorbic acid, 1 M NaCl. Peroxidases reacted with gaiacol 55 mM and its enzymatic activity was measured by absorption spectrometry at 470 nm every second for 20 s using a spectrophotometer (JENWAY 7305). 2 g of sample were dissolved in 0.18 mM citric acid, 16 mM Na2HPO4, PVPP 10%. Polyphenoloxidase reacted with catechol and its enzymatic activity was measured by absorption spectrometry at 400 nm every second for 30 s using a spectrophotometer (JENWAY 7305). Lipoxigenase activity was determined using a spectrophotometer (JENWAY 7305).

**Vitamin C:** Vitamin C was measured using the K-ASCO 11/05 Megazyme kit. This is a colorimetric method and the absorbance at 578 nm was read using a spectrophotometer (JENWAY 7305).

**Vitamin B3:** Vitamin B3 was determined with the EN 15562 2009 method.

**Cyanohydrac acid:** Cyanohydrac acid determinations were undertaken using the HS-GC-NPD method.

**Sensorial analyses**

Sweet cassava samples were cleaned, peeled, bleached, placed in plastic bags, frozen and warmed just before sensorial analyses. Twelve individuals were selected as panel members, all of them inexperienced in sensory analysis. Five 2-h training sessions were carried out until they were able to recognize and rate the characteristics of different cassava varieties according to the AFNOR 8586-1 and 8586-2 standards. Fifteen (15) attributes represented the sensory profile according to the AFNOR 13299 standard. Significant differences (p<0.05) were found for the 15 sensory attributes. Homogeneity in the group was also tested by two-way analysis of variance (ANOVA) for each attribute and the training was considered complete when no panellists × samples interaction was found. ANOVA were carried out with FIZZ®, UNIWIN Plus v6.1 and StatGraphics CENTURION® XV 2005 software with a confidence interval of 5%.

**Data statistical analysis**

The physical and chemical mean values of triplicate measurements or analysis were statistically analysed. ANOVA, based on student tests, principal component analysis (PCA) and Duncan’s multiple range test (DMRT) were performed using the software StatGraphics CENTURION® XV 2005 and Uniwin PLUS® v6.1.

**RESULTS**

**Agronomic data**

The first step was a survey and screening of varieties by the local department of agriculture throughout the region of Martinique. Phenotypic characterization and identification were then established. Sweet cassavas were grown on contiguous plots and in the same agro-climatic conditions for a minimum of nine months. Cropping system data was collected using a delivery sheet, provided by the department of agriculture, and is summarized in Table 1. Cultivars ‘KM06’, ‘KM07’ and ‘KM08’ exhibited phenotypic differences such as colour of the petiole. ‘KM06’ and ‘KM08’ had the same flesh colour. ‘KM08’ is distinguished by a pink-coloured cortex. ‘KMRA’
Table 1. Cropping system data of the four sweet cassava varieties studied.

<table>
<thead>
<tr>
<th>Variety</th>
<th>KM06</th>
<th>KM07</th>
<th>KM08</th>
<th>KMRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average rainfall (mm)</td>
<td>1900 to 2000 mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irrigation</td>
<td>in extra</td>
<td>in extra</td>
<td>in extra</td>
<td>no use of water</td>
</tr>
<tr>
<td>Soil type</td>
<td>Ferralitic</td>
<td></td>
<td></td>
<td>Sandy and pumice soil</td>
</tr>
<tr>
<td>Previous crop</td>
<td>Fallow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>No fungicide, insecticide and nematicide</td>
<td>Chemical weed control (glyphosate) around</td>
<td>Search chlordecone negative</td>
<td>Manual weeding</td>
</tr>
<tr>
<td></td>
<td>Weeding at will</td>
<td>Satisfactory level of fertility</td>
<td></td>
<td>No fungicide, insecticide and nematicide</td>
</tr>
<tr>
<td>Observations</td>
<td>Dense vegetation</td>
<td>Moderately dense vegetation</td>
<td>Very dense vegetation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plant height: 2.5 m</td>
<td>Plant height: 2 m</td>
<td>Plant height: 2.5 m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flesh colour: white</td>
<td>Flesh colour: yellow</td>
<td>Flesh colour: white</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Petiole colour: green</td>
<td>Petiole colour: green</td>
<td>Petiole colour: red</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parchment/cortex colour: white</td>
<td>Parchment/cortex colour: white</td>
<td>Parchment/cortex colour: pink</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Average scores out of 7 for sensorial analysis descriptors of the four sweet cassava varieties.

<table>
<thead>
<tr>
<th>Sensory attribute</th>
<th>KM06</th>
<th>KM07</th>
<th>KM08</th>
<th>KMRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour***</td>
<td>3.08 ± 1.71b</td>
<td>5.54 ± 1.33a</td>
<td>3.00 ± 1.47b</td>
<td>3.46 ± 1.56b</td>
</tr>
<tr>
<td>Colour homogeneity</td>
<td>5.62 ± 1.33</td>
<td>5.62 ± 0.87</td>
<td>5.38 ± 1.04</td>
<td>5.15 ± 0.99</td>
</tr>
<tr>
<td>Odour</td>
<td>4.31 ± 1.11</td>
<td>4.62 ± 1.12</td>
<td>4.77 ± 1.30</td>
<td>4.77 ± 1.01</td>
</tr>
<tr>
<td>Taste</td>
<td>4.46 ± 1.33</td>
<td>4.38 ± 1.45</td>
<td>4.38 ± 1.66</td>
<td>4.85 ± 1.21</td>
</tr>
<tr>
<td>Earthy taste</td>
<td>2.85 ± 1.57</td>
<td>3.00 ± 1.78</td>
<td>3.15 ± 1.68</td>
<td>3.08 ± 1.75</td>
</tr>
<tr>
<td>Saltiness</td>
<td>3.15 ± 1.21</td>
<td>2.92 ± 1.12</td>
<td>3.08 ± 0.86</td>
<td>3.15 ± 1.34</td>
</tr>
<tr>
<td>Bitterness***</td>
<td>2.69 ± 1.55bc</td>
<td>2.08 ± 1.50c</td>
<td>4.15 ± 1.41a</td>
<td>3.00 ± 1.68b</td>
</tr>
<tr>
<td>Astringency</td>
<td>2.85 ± 1.77</td>
<td>2.85 ± 1.46</td>
<td>3.23 ± 1.42</td>
<td>3.00 ± 1.68</td>
</tr>
<tr>
<td>Firm texture**</td>
<td>4.62 ± 1.19ab</td>
<td>5.15 ± 1.21a</td>
<td>3.62 ± 1.39b</td>
<td>3.85 ± 1.14bc</td>
</tr>
<tr>
<td>Mealy texture</td>
<td>5.00 ± 1.19</td>
<td>4.46 ± 1.51</td>
<td>4.46 ± 1.05</td>
<td>5.38 ± 1.26</td>
</tr>
<tr>
<td>Fibrous texture</td>
<td>3.08 ± 1.66</td>
<td>3.46 ± 1.85</td>
<td>3.62 ± 1.39</td>
<td>3.08 ± 1.71</td>
</tr>
<tr>
<td>Sticky texture</td>
<td>4.62 ± 1.04</td>
<td>3.62 ± 1.50</td>
<td>4.08 ± 1.38</td>
<td>4.23 ± 1.01</td>
</tr>
<tr>
<td>Melting texture**</td>
<td>3.77 ± 1.36ab</td>
<td>2.92 ± 1.32b</td>
<td>4.62 ± 1.56a</td>
<td>3.69 ± 1.25ab</td>
</tr>
</tbody>
</table>

*Significant descriptors at 5%; **Significant descriptors at 1%; ***Significant descriptors at 0.1%; NS, No significant. Different letters (a-c) within the same line indicate significant differences at (p<0.05), using Duncan's multiple-range test.

Tubers had a phenotypic appearance similar to 'KM06'.

Sensorial analysis

A sensorial analysis was carried out on the four cassavas. Thirteen (13) descriptors were selected. Table 2 shows the results with the average scores out of seven for these descriptors. An analysis of variance was performed to evaluate the organoleptic characteristics and differences between the samples of cassavas presented to specialized juries, and for each descriptor. The interpretation of results can highlight significant descriptors, descriptors which reveal a statistically reliable difference between the samples. In contrast, no difference between the samples can be established for insignificant descriptors. The statistical analysis of this data reveals that 4 of the 13 descriptors evaluated varied significantly: colour, bitterness, firm texture and melting texture (Table 2). For the other criteria evaluated, no significant differences were found in the four varieties of cassavas. Their specific taste and smell, astringency and mealy and sticky mouth texture were comparable. They
had the following general characteristics: a consistent colour, a moderate overall intense odour, overall a slightly earthy, bitter, salty, astringent taste, a firm mealy, slightly sticky, fibrous and melting texture. A profile was established for each cultivar and presented in Figure 1. We noted, however, that the intense mealy mouth texture tended to be different (5.86%) between samples. Cassava ‘KM07’ differed significantly from the other four cultivars by its more intense colour, slightly bitter taste and firm and slightly sticky mouth texture. Conversely, the ‘KM08’ cultivar was distinguished by a more intense bitterness, slightly firm and very smooth mouth feel. Cassava ‘KM06’ was statistically comparable to ‘KMRA’. Both cultivars were placed in the middle position for these four criteria. With regard to mealy mouth textures, ‘KM06’ and ‘KMRA’ cultivars tended to have the highest intensity.

Technological characteristics

All samples were weighed and measured to determine their calibre. The results are shown in Table 3. ANOVA was performed on the data obtained (diameter, length and weight) to highlight the features and significant differences observed for each class of samples. Analysis of the results revealed that, in terms of size, varieties differed statistically by 5% for their diameter (p = 0.0034). Multiple range tests (method of Fischer minimum significant differences (LSD), 95% confidence level) highlights three groups. ‘KM08’ had the smallest diameter and ‘KMRA’ the largest. ‘KM06’ and ‘KM07’ were in an intermediate position. We noted, however, in terms of weight, that the cultivar ‘KM08’ tended (p = 0.07) to be lighter than the cultivar ‘KM06’. Other cassavas belonged to the same statistical group and were placed in an intermediate position. Figure 2 shows average trans-formation yields of the four samples of cassava. The four cultivars of cassava had close average cooking yields (~ 91 ± 1%). The four cultivars differed in their peel yield; ‘KM06’ and ‘KM07’ samples were close, with lower peel yields. Conversely, ‘KM08’ had the highest peel yield (86%). Finally, the overall flesh yield had the same profile as the peel yield. This yield was average for ‘KM06’ and ‘KM07’ cultivars, with 58%. It was highest for ‘KM08’.

Texture analyses were performed on the four cooked slices of cassava samples. The results of the five texture parameters studied are shown in Table 3. ANOVA was performed to highlight the characteristics and significant texture differences observed for each sample. This analysis of the different varieties revealed that four texture parameters of the five evaluated differed significantly between cultivars. ‘KM08’ cultivar was characterized by lower hardness and cohesion and higher tensile strength than other cultivars. Cassava ‘KMRA’ demonstrated higher chewiness values than other cultivars. ‘KM07’ was significantly harder. Finally, ‘KM06’ was in an intermediate position for all parameters evaluated. Texture results corroborate the data obtained by sensory analysis, including hardness values. Indeed, we observed the same profile for ‘KM07’ and ‘KM08’, that is, firmer and less firm, respectively.

The colour of a product according to the parameters
Table 3. Physical parameters of the four sweet cassava varieties.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Length (cm)</th>
<th>Diameter (cm)</th>
<th>Weight (g)</th>
<th>Hardness (kgf)</th>
<th>Cohesion (kgf.mm)</th>
<th>Elasticity (kgf.mm)</th>
<th>Chewiness (kgf.mm)</th>
<th>Tensile strength (kgf)</th>
<th>L</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>KM06</td>
<td>29.60 ± 10.58</td>
<td>5.26 ± 1.19</td>
<td>531.67 ± 304.61</td>
<td>5.91 ± 1.39</td>
<td>0.06 ± 0.02</td>
<td>11.48 ± 2.25</td>
<td>5.79 ± 2.23</td>
<td>0.03 ± 0.02</td>
<td>81.96 ± 0.17</td>
<td>-0.16 ± 0.02</td>
<td>23.41 ± 0.20</td>
</tr>
<tr>
<td>KM07</td>
<td>27.95 ± 11.76</td>
<td>4.57 ± 0.66</td>
<td>376.77 ± 200.29</td>
<td>6.31 ± 1.55</td>
<td>0.07 ± 0.03</td>
<td>12.69 ± 0.55</td>
<td>5.56 ± 1.47</td>
<td>0.03 ± 0.01</td>
<td>78.61 ± 0.05</td>
<td>-1.34 ± 0.02</td>
<td>46.75 ± 0.03</td>
</tr>
<tr>
<td>KM08</td>
<td>26.8 ± 5.56</td>
<td>4.41 ± 0.59</td>
<td>274.95 ± 135.23</td>
<td>2.59 ± 1.00</td>
<td>0.15 ± 1.00</td>
<td>13.05 ± 0.04</td>
<td>4.60 ± 1.26</td>
<td>0.05 ± 0.01</td>
<td>82.1 ± 0.23</td>
<td>-0.21 ± 0.01</td>
<td>22.35 ± 0.09</td>
</tr>
<tr>
<td>KMRA</td>
<td>26.65 ± 4.81</td>
<td>5.77 ± 0.61</td>
<td>467.2 ± 130.44</td>
<td>4.79 ± 1.03</td>
<td>4.79 ± 1.03</td>
<td>13.46 ± 4.18</td>
<td>11.07 ± 6.19</td>
<td>0.02 ± 0.01</td>
<td>108.04 ± 10.23</td>
<td>3.88 ± 1.02</td>
<td>32.89 ± 5.16</td>
</tr>
</tbody>
</table>

$L^*$, $a^*$ and $b^*$ results in its positioning in three-dimensional space. Values were measured on cooked slices of the four cassava cultivars (Table 3). ANOVA was performed to bring out the characteristics and statistical colour differences observed for each variety. This analysis revealed that the three colour parameters varied by 5%. The parameter $L^*$ defines the brightness of product: 0 black, 100 white. There was a statistically
The hydrocyanic acid content of all cultivars was found to be below 0.5 mg/kg. According to Codex Alimentarius (CX/CF 03/09/11 December 2008), varieties can be defined as sweet cassava if their cyanide hydrogen content is less than 50 mg/kg. ‘KM06’ and ‘KM07’ cultivars have similar energy values, carbohydrates and dry matter and show the highest values. ‘KM08’ had the lowest levels for these three parameters. ‘KMRA’ offered the highest levels of total ash (1.2%) and starch (20.15 g/100 g). ‘KM07’ also presented high starch content compared to ‘KMRA’ but the lowest level of total ash.

Overall, all four cassava cultivars had low levels of vitamin B3. ‘KM07’ had the highest level of vitamin C (29.8 mg/100 g) and total polyphenols (value 49.95 mg of GAE/100 g), and the lowest fibre content. Levels of vitamin C for ‘KM06’ and ‘KMRA’ were similar (statistically significant). ‘KM08’ was also a source of vitamin C (14.3 mg/100 g). The highest fibre content was found in the ‘KMRA’ cultivar, which is classed as rich in fibre (> 3 g/100 g). Finally, all cultivars' total polyphenols content were higher than 34 mg of GAE/100 g. ‘KM06’ had the lowest content of total polyphenols (34.8 mg of GAE/100 g). Mineral content was highly variable depending on cultivar; values were generally low. ‘KMRA’ was the only source of potassium with 327.91 mg/100 g. ‘KM07’ also presented high phosphorus and total ash. The highest fibre content was found in the ‘KMRA’ cultivar, which is classed as rich in fibre (> 3 g/100 g). Finally, all cultivars' total polyphenols content were higher than 34 mg of GAE/100 g. ‘KM06’ had the lowest content of total polyphenols (34.8 mg of GAE/100 g). Mineral content was highly variable depending on cultivar; values were generally low. ‘KMRA’ was the only source of potassium with 327.91 mg/100 g. ‘KM07’ had the highest content of total ash (1.2%) and starch (20.15 g/100 g). ‘KM07’ also presented high starch content compared to ‘KMRA’ but the lowest level of total ash.

Overall, all four cassava cultivars had low levels of vitamin B3. ‘KM07’ had the highest level of vitamin C (29.8 mg/100 g) and total polyphenols (value 49.95 mg of GAE/100 g), and the lowest fibre content. Levels of vitamin C for ‘KM06’ and ‘KMRA’ were similar (statistically significant). ‘KM08’ was also a source of vitamin C (14.3 mg/100 g). The highest fibre content was found in the ‘KMRA’ cultivar, which is classed as rich in fibre (> 3 g/100 g). Finally, all cultivars' total polyphenols content were higher than 34 mg of GAE/100 g. ‘KM06’ had the lowest content of total polyphenols (34.8 mg of GAE/100 g). Mineral content was highly variable depending on cultivar; values were generally low. ‘KMRA’ was the only source of potassium with 327.91 mg/100 g. ‘KM07’ had the highest content of total ash (1.2%) and starch (20.15 g/100 g). ‘KM07’ also presented high starch content compared to ‘KMRA’ but the lowest level of total ash.

Overall, all four cassava cultivars had low levels of vitamin B3. ‘KM07’ had the highest level of vitamin C (29.8 mg/100 g) and total polyphenols (value 49.95 mg of GAE/100 g), and the lowest fibre content. Levels of vitamin C for ‘KM06’ and ‘KMRA’ were similar (statistically significant). ‘KM08’ was also a source of vitamin C (14.3 mg/100 g). The highest fibre content was found in the ‘KMRA’ cultivar, which is classed as rich in fibre (> 3 g/100 g). Finally, all cultivars' total polyphenols content were higher than 34 mg of GAE/100 g. ‘KM06’ had the lowest content of total polyphenols (34.8 mg of GAE/100 g). Mineral content was highly variable depending on cultivar; values were generally low. ‘KMRA’ was the only source of potassium with 327.91 mg/100 g. ‘KM07’ had the highest content of total ash (1.2%) and starch (20.15 g/100 g). ‘KM07’ also presented high starch content compared to ‘KMRA’ but the lowest level of total ash.

### Physicochemical analyses

Nutritional analyses were performed on cooked products. Table 4 summarizes the results of measured nutritional parameters. The hydrocyanic acid content of all cultivars studied is less than 0.5 mg/kg. According to Codex Alimentarius (CX/CF 03/09/11 December 2008), varieties can be defined as sweet cassava if their cyanide hydrogen content is less than 50 mg/kg. 'KM06' and 'KM07' cultivars have similar energy values, carbohydrates and dry matter and show the highest values. 'KM08' had the lowest levels for these three parameters. 'KMRA' offered the highest levels of total ash (1.2%) and starch (20.15 g/100 g). 'KM07' also presented high starch content compared to 'KMRA' but the lowest level of total ash.

Overall, all four cassava cultivars had low levels of vitamin B3. 'KM07' had the highest level of vitamin C (29.8 mg/100 g) and total polyphenols (value 49.95 mg of GAE/100 g), and the lowest fibre content. Levels of vitamin C for 'KM06' and 'KMRA' were similar (statistically significant). 'KM08' was also a source of vitamin C (14.3 mg/100 g). The highest fibre content was found in the 'KMRA' cultivar, which is classed as rich in fibre (> 3 g/100 g). Finally, all cultivars' total polyphenols content were higher than 34 mg of GAE/100 g. 'KM06' had the lowest content of total polyphenols (34.8 mg of GAE/100 g). Mineral content was highly variable depending on cultivar; values were generally low. 'KMRA' was the only source of potassium with 327.91 mg/100 g. 'KM07' had the highest content of total ash (1.2%) and starch (20.15 g/100 g). 'KM07' also presented high starch content compared to 'KMRA' but the lowest level of total ash.

### Table 4. Nutritional composition of the four sweet cassava varieties (Abbreviation used: GAE: Gallic Acid Equivalent).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>KM06</th>
<th>KM07</th>
<th>KM08</th>
<th>KMRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>55.28</td>
<td>54.72</td>
<td>65.97</td>
<td>59.5</td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td>44.73</td>
<td>45.28</td>
<td>34.03</td>
<td>40.5</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1</td>
<td>0.93</td>
<td>1.04</td>
<td>1.2</td>
</tr>
<tr>
<td>Carbohydrates (g/100 g)</td>
<td>42.45</td>
<td>42.82</td>
<td>31.27</td>
<td>38.64</td>
</tr>
<tr>
<td>Starch (g/100 g)</td>
<td>8.64</td>
<td>18.78</td>
<td>7.44</td>
<td>20.15</td>
</tr>
<tr>
<td>Proteins (g/100 g)</td>
<td>1</td>
<td>1.34</td>
<td>1.57</td>
<td>0.56</td>
</tr>
<tr>
<td>Lipids (g/100 g)</td>
<td>0.29</td>
<td>0.2</td>
<td>0.16</td>
<td>0.13</td>
</tr>
<tr>
<td>VE (Kcal/100 g)</td>
<td>176.35</td>
<td>178.45</td>
<td>132.75</td>
<td>157.9</td>
</tr>
<tr>
<td>Total polyphenols (mg of GAE/100 g)</td>
<td>34.8</td>
<td>49.95</td>
<td>41.8</td>
<td>36.75</td>
</tr>
<tr>
<td>Fibres (g/100g)</td>
<td>2.5</td>
<td>2.4</td>
<td>2.9</td>
<td>3.6</td>
</tr>
<tr>
<td>Vitamin C (mg/100 g)</td>
<td>17.4</td>
<td>29.8</td>
<td>14.3</td>
<td>18.35</td>
</tr>
<tr>
<td>Vitamin B3 (mg/100 g)</td>
<td>0.898</td>
<td>0.937</td>
<td>0.837</td>
<td>0.734</td>
</tr>
<tr>
<td>P (mg/100 g)</td>
<td>1.62</td>
<td>2.65</td>
<td>2.7</td>
<td>2.94</td>
</tr>
<tr>
<td>Ca (mg/100 g)</td>
<td>32.15</td>
<td>25.17</td>
<td>17.97</td>
<td>18.4</td>
</tr>
<tr>
<td>K (mg/100 g)</td>
<td>285.95</td>
<td>260.1</td>
<td>225.54</td>
<td>327.91</td>
</tr>
<tr>
<td>Zn (mg/100 g)</td>
<td>0.16</td>
<td>0.38</td>
<td>0.25</td>
<td>0.21</td>
</tr>
<tr>
<td>Mg (mg/100 g)</td>
<td>1.12</td>
<td>0.65</td>
<td>0.45</td>
<td>0.55</td>
</tr>
<tr>
<td>HCN (mg/kg)</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>PPO (AU/s/100 g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peroxidase (Au/s/100 g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lipoxigenase (Au/s/100 g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>pH</td>
<td>5.94</td>
<td>6.25</td>
<td>6.06</td>
<td>5.94</td>
</tr>
</tbody>
</table>
Table 5. Comparison between the average nutritional composition of the four cultivars of sweet cassava studied and an average sweet cassava studied globally.

<table>
<thead>
<tr>
<th>Nutritional composition</th>
<th>Average nutritional composition of the four sweet cassavas studied</th>
<th>Average data for sweet cassavas - international nutritional composition tables</th>
<th>Average data for potatoes - international nutritional composition tables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (% )</td>
<td>58.87 ± 3.87</td>
<td>60.07 ± 2.23</td>
<td>78.9 ± 3.36</td>
</tr>
<tr>
<td>Ash (g/100 g)</td>
<td>1.04 ± 0.08</td>
<td>0.92 ± 0.32</td>
<td>0.70 ± 0.28</td>
</tr>
<tr>
<td>Energy value (kcal)</td>
<td>161.36 ± 16.04</td>
<td>150.43 ± 11.62</td>
<td>81.60 ± 9.05</td>
</tr>
<tr>
<td>Proteins (g/100 g)</td>
<td>1.12 ± 0.34</td>
<td>1.12 ± 0.19</td>
<td>2.20 ± 0.43</td>
</tr>
<tr>
<td>Lipids (g/100 g)</td>
<td>0.20 ± 0.05</td>
<td>0.27 ± 0.11</td>
<td>0.26 ± 0.06</td>
</tr>
<tr>
<td>Carbohydrates (g/100 g)</td>
<td>38.80 ± 3.84</td>
<td>36.15 ± 2.34</td>
<td>17.80 ± 1.80</td>
</tr>
<tr>
<td>Fibres (g/100 g)</td>
<td>2.85 ± 0.40</td>
<td>1.91 ± 0.55</td>
<td>2.05 ± 0.62</td>
</tr>
<tr>
<td>Starch (g/100 g)</td>
<td>13.75 ± 5.71</td>
<td>-</td>
<td>15.8 ± 1.45</td>
</tr>
</tbody>
</table>

Minerals

| K (mg/100 g) | 274.88 ± 32.06 | 297.60 ± 32.84 | 395.33 ± 108.21 |

Vitamins

| Vitamin C (mg/100 g) | 19.96 ± 4.92 | 28.47 ± 5.22 | 18.52 ± 9.51 |
| Niacin (B3) (mg/100 g) | 0.85 ± 0.07 | 0.67 ± 0.16 | 1.74 ± 0.15 |

...for its contents of phosphorus and zinc. It had the lowest calcium, potassium and manganese contents. 'KMRA' had the highest content of potassium and phosphorus of the four cultivars, with 327.91 and 2.94 mg/100 g respectively. The enzymatic activity of four cultivars was null for the three enzymes studied, polyphenoloxidase, peroxidase and lipoxygenase. The average pH of the four varieties is about 6.4 ± 0.12.

DISCUSSION

All sweet cassavas studied in Martinique were rich in minerals, vitamin C and polyphenols. Vitamin C is known for its antioxidant properties (Padayatty et al., 2003). Vitamin B3 values were also high. Vitamin B3, also called niacin, is most frequently found in meat. It plays an important role in the release of energy from food, the functioning of the nervous system and also reduces cholesterol (Sauve, 2008). Total polyphenols are the most abundant antioxidants in our diet and foods rich in polyphenols are thought to play a role in the prevention of certain diseases (Scalbert et al., 2005). Therefore, based on these results and the vitamin and mineral contents observed, it can be argued that Martiniquan sweet cassavas have numerous health benefits (Sarkiyari and Agar, 2010).

The total polyphenol content and polyphenol oxidase activity lead to enzymatic browning. The higher the content or activity, the greater the sensitivity to enzymatic browning. Polyphenol oxidase is an enzyme with an essential role in the oxidation of phenolic compounds. In the presence of oxygen, it is responsible for the transformation of polyphenol into coloured compounds. Polyphenol oxidase is therefore a good indicator of enzymatic browning. It can be inhibited by exposure to high temperatures for short durations (bleaching). Visually, browning of the flesh was not observed during cassava processing (during peeling, cooking and whilst awaiting treatment). It remained the same throughout processing. This correlates with the enzymatic activities of three enzymes, which are null. Brightness has been identified as a factor influencing results. Indeed, it relates to the lighter or darker appearance of a product. The higher the value, the lighter the product and vice versa. Factors a * and b * express the colour itself and therefore were not of interest here in translating enzymatic browning. Results were therefore obtained only from the factor L *. We did not observe any change in the value of L * over time. This also correlates with the absence of enzyme activity and no visual observation of browning of cassava flesh during treatment.

Table 5 compares the average nutritional composition of the four varieties studied to the nutritional composition of sweet cassava averages based on international tables of compositions (USA, France, Germany, South Africa, Denmark, Finland, Australia, Pacific and Brazil). The four varieties grown in Martinique were advantageously positioned in relation to the globally identified data for their energy values, proteins, lipids, carbohydrates, fibre, vitamin C and niacin (vitamin B3) content. Conversely, their potassium content is lower than the average sweet cassava worldwide. Compared to potatoes, sweet cassavas in Martinique have greater nutritional potential. Their energy value was almost twice as much as for normal potatoes. Sweet cassavas also contained more...
vitamin C and fewer lipids than potatoes.

In comparison with other vegetables for the rate of total polyphenols (Brat et al., 2006), sweet cassava would be in twelfth position, with about 40.82 mg of GAE/100 g on average, before potatoes and peas (respectively, 23.1 and 36.1 mg of GAE/100 g). We attempted to demonstrate different ways of using sweet cassavas (fresh and processed). We selected specific criteria to conduct this analysis (size / shape, sensory profile, nutritional value, average yield of transformation). Sweet cassavas with small size and regular shape are best for selling directly to consumers (purchase volume, ease of cutting and peeling). Cultivars with uniform colour, firm and / or melting textures and high nutritional potential also have greater commercial potential in relation to consumers. Conversely, sweet cassavas with large, irregular shapes, fibrous appearance, granular or powdery texture and / or low nutritional properties are more suitable for industrial uses (flour, mashed, flakes). Finally, when cultivars are characterized by intermediate criteria, they can be used both as a fresh product or processed. Thus, ‘KM06’ is most suitable for processed products because of its large size, very firm, mealy and sticky texture (very unpopular with consumers) and nutritional characteristics - mineral levels (high K and Ca contents), carbohydrates and dry matter content. ‘KM07’ displays criteria that make it suitable for fresh consumption or processing; its small size, regular shape, colour and nutritional properties (high vitamin C, carbohydrates and dry matter content) make it suitable for fresh consumption, whilst high dry matter content and texture make it suitable for processing into flour or frozen products.

Variety ‘KM07’ therefore seemed to offer the best compromise between size and nutritional and sensory characteristics and it would thus be recommended for production. In contrast, ‘KM08’ would be better for processed products because of its slightly firm and melting texture and low nutritional potential, including vitamin and mineral levels. Its small size and high concentration of polyphenols are the only criteria in its favour in terms of fresh consumption. The regular form of ‘KMRA’, together with its starch and high mineral content, give it greater value. However, it is very fibrous and lacks texture, which may be considered a disadvantage. Conducting consumer tests on these cultivars will determine their acceptability criteria with relation to consumers in Martinique.

Conclusions

This study is the first in investigating varietal characterization of four sweet cassavas in Martinique. It is also one of the first study of the cassava variety *M. opii* and the variety *M. esculenta* Crantz is one of its primary areas of focus. The four cultivars were distinguished by their phenotypic, sensory, physicochemical and nutritional characteristics. ‘KMRA’ cultivar was distinguished by its cultivation techniques but had the same phenotypic characteristics as ‘KM06’. There was no significant difference between the lengths of cassavas, but varieties differed significantly with respect to their colour. ‘KMRA’ was the brightest and ‘KM07’ had a rather yellow tint. ‘KM08’ was significantly narrower, had the best average yield of transformation and the lowest carbohydrate content. ‘KM06’ was also less firm but had a cohesion and tensile strength significantly higher than other cultivars. ‘KM07’ cultivar was the hardest and had the highest total polyphenol content. ‘KMRA’ rated highest for chewiness. ‘KM06’ had the lowest total polyphenol content. Martinique sweet cassavas are richer in nutritional compounds and vitamin C than potatoes. Sweet cassavas also have a lower glycaemic index, which means they could be of interest for individuals affected by obesity and diabetes. Fact sheets were developed for each studied cultivar combining all the data obtained, as well as recommendations in terms of value and usefulness of each cultivar. Their sensory specificity, high nutritional value and suitability for industrial processing have also been highlighted. In view of their potential in terms of consumption and processing, further investigations into the qualities of these tubercles are needed. Further studies could aid in highlighting their positive characteristics and thus encourage further development within professional agriculture and agribusinesses. A cassava research-for-development program was carried out in the Democratic Republic of Congo and highlighted impact at the farm level (Rusike et al., 2014). This work is thus especially helpful for optimal management of crop harvesting in a region of agriculture and climatic contrasts.

Conflict of Interests

The author(s) have not declared any conflict of interest.

REFERENCES


Seed pre-treatment methods for improving germination of *Acacia tortilis*

So Hanaoka¹*, Norio Nakawa¹, Norihisa Okubo¹, Stephen Fredrick Omondi² and Jason Kariuki²

¹Forest Tree Breeding Center, Forestry and Forest Products Research Institute, 3809-1 Ishi, Hitachi City, Ibaraki, 319-1301, Japan.
²Kenya Forestry Research Institute, P.O. Box 20412 – 00200, Nairobi, Kenya.

Received 6 October, 2014; Accepted 2 December, 2014

*Acacia tortilis* (Forsk.) Hayne is considered as an important dryland tree species in Africa and Middle East, and establishing an effective and efficient seed-germination method is considered necessary for producing planting materials. In this study, new germination method was compared with some of the previously reported methods and evaluated in *A. tortilis*. In the new method, root setting was observed within 3 days and the final germination percentage was 94%; higher than 5 to 86% reported in the previous methods. New method is therefore considered to be suitable for gerninating *A. tortilis* seeds.

Key words: *Acacia tortilis*, seed germination, pre-treatment of seed germination.

INTRODUCTION

*Acacia tortilis* (Forsk.) Hayne (subfamily Mimosoideae, family Leguminosae) is one of approximately 135 African acacia species. The morphology of *A. tortilis* varies considerably, ranging from multi-stemmed shrubs (ssp. tortilis) to trees measuring up to 20 m in height with rounded (ssp. raddiana) or flat-topped (ssp. heteracantha and spirocarpa) crowns. The species is widely distributed throughout the Middle East and south into eastern and southern Africa, in habitats ranging from semi-desert to dry bushland and grassland (Allen, 2007; Hines and Eckman, 1993). In Africa, *A. tortilis* occurs in the drier parts of Senegal and Somalia and extends all the way down to South Africa. The species is highly drought resistant and grows in areas with annual rainfall ranging from as low as 40 mm to as much as 1200 mm, with dry seasons lasting 1-12 months. *A. tortilis* tree can be used for a variety of purposes. The pods, which are high in protein (15-20%), fall to the ground where they are eaten by livestock and wildlife. Leaves, new shoots, and seedlings are also eaten, with leaves being available throughout most of the dry season when other fodder sources are scarce. The gum is edible, and the bark can be used to produce strong fibre. *A. tortilis* starts producing firewood at 8-18 years, at a rate of 50 kg/tree/year. Its fast growth and good coppicing ability, coupled with the high calorific value of its wood of about

*Corresponding author. E-mail: sohana@affrc.go.jp. Tel: +81-294-39-7013. Fax: +81-294-39-7306.

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License.
4400-4900 kcal/kg, make it well suited for use as firewood and charcoal (Orwa et al., 2009; National Academy of Sciences, 1980). Given the economic value of this species, efficient and stable seedling production is important. However, several challenges exist regarding artificial seed germination.

Acacia species generally have a water-impermeable seed coat, which means that physical and/or chemical pre-germination treatments are necessary in order to overcome seed dormancy and obtain rapid and synchronous germination. For example, soaking seeds in boiling water, sulphuric acid, or mechanical scarification treatments have been demonstrated to promote germination in Acacia species (Danthu et al., 1992; Teketay, 1998; Aref, 2000; Rodrigues et al., 2008). As with other Acacia species, the seeds of A. tortilis also have a hard seed coat and pre-treatment is essential to overcome seed dormancy. Loth et al. (2005) indicated that ingestion by herbivores or stimulation by fire may promote germination of A. tortilis seeds under natural conditions. In addition, the study also showed that a wet environment and optimal temperature conditions (from 15 to 25°C) is suitable for germination of the species. More recently, several studies have demonstrated varying levels of success in promoting A. tortilis seed germination. For example, Abari et al. (2012) reported a germination percentage of around 80% through scarification of the seed coat or combined use of sulphuric acid and hydrogen peroxide. However, the germination percentage after treatment with sulphuric acid alone was low in the study. On the other hand, Azazi et al. (2013) tested a number of chemical treatments and found that boiling in water or treatment with sulphuric acid for more than 20 min were both comparatively efficient, and the germination percentages obtained with these treatments was approximately 75%.

In addition, a germination percentage of 52% was obtained after mechanical scarification. Although both studies concluded that mechanical scarification, immersion in boiling water or sulphuric acid were all effective, the obtained germination percentages were relatively variable. For the sulphuric acid treatment, differences in immersion duration may have accounted for the observed differences in germination percentages in both studies, with immersion for a minimum of 20 min associated with optimal results. However, in addition to being potentially dangerous and unsafe for local farmers, chemical treatments are typically difficult to achieve in rural areas. Regarding treatments involving boiling water or mechanical scarification, it is considered likely that slight differences in experimental conditions might affect germination percentages. If such condition-dependent differences do exist, then it may be difficult for local farmers to repeat experimental conditions and obtain the same the results. In this paper, we report and discuss simpler, safer and more efficient method for improving germination percentages in A. tortilis seeds.

**MATERIALS AND METHODS**

Seed materials and pre-treatments

Seeds of A. tortilis were collected from several mother trees in Kilwezi, Kenya. The seeds were processed, bulked, and sent to the Forest Tree Breeding Centre (FTBC) in Japan where they were stored at room temperature for two months until the following experiments were initiated: Treatment 1 (Cut-Hilum): The seeds of A. tortilis are flattened and ovoid in shape, with a white hilum on the seed coat (Figure 1a). We defined the surface with hilum as the "upper side", which we removed by nail scissors (Figure 1b); Treatment 2 (Cut-Side): The seed coat of the "lower side" was removed by nail-scissors. Treatment 3 (Cut-Bot): Seed coat of the "side" (that is, the seed coat between the upper and lower sides) was removed using nail scissors; Treatment 4 (Scr-Bot): The seed coat was first scratched using sandpaper and then placed in boiling water at 98°C. The boiling water was allowed to cool naturally to room temperature following Abari et al (2012); Treatment 5 (Bo): Without removing or scratching the seed coat, seeds were placed in boiling water at 98°C. The boiling water was allowed to cool naturally to room temperature; Treatment 6 (Scr-Sul): The seed coat was scratched using sandpaper, before being immersed in concentrated sulphuric acid solution for 20 min and washed in distilled water; Treatment 7 (Sul): Seeds were immersed in concentrated sulphuric acid solution for 20 min and washed in distilled water; Treatment 8 (Scr): Seed coat was scratched using sandpaper; Treatment 9 (Cont): No treatment (control).

Mechanical scarification of A. tortilis seeds using sandpaper is complicated by small size of the seeds and the hardness of the coat. We therefore only scratched the surface of the seed coat and did not produce deep scars on the coat. Treatments 1 to 3 are unique to this study while treatments 4 to 8 were based on previous studies (Abari et al., 2012; Azazi et al., 2013).

Comparison of the time to root production among treatments

Fifty seeds were used in each of the nine treatments described above. After completing each treatment, the seeds were placed in water-filled Petri dishes and root setting (produced a root of > 2 mm) was observed every morning at 8:30 am for 14 days. We also identified which seeds absorbed water within the first 3 days. However, the presence of mould on some seeds at day 15, meant that the experiments had to be stopped. Expected cumulative rate of root setting over time and their 95% confidence interval were calculated by the Kaplan Meier method (Measurement of the effect of intervention over a period of time, sometimes called "time-to-event analysis"). In addition, the differences in rate of root setting among the treatments were tested by Log rank test. Seeds with roots were planted in soil containing compost and their rates of cotyledon establishment were confirmed and noted. The experiments were performed in a greenhouse at a mean temperature of 27.9±6.9°C.

Germination percentage of seeds in each treatment

In the above experiments, observations were stopped on the 15th day. To examine germination percentages over an extended period of time, 100 seeds were planted in sand at a depth of 10 mm after each of the above treatments. Because we understood that treatment 1 was more efficient than treatment 2 and 3 in above experiments, we did not try treatment 2 and 3 in this experiment. Watering was performed once every two to three days to prevent seeds from drying. The number of seedlings was counted on the 28th day and germination percentage was calculated as: (numbers of germinated seed / numbers of total seeds examined). The
experiments were performed in a greenhouse at a mean temperature of 23.9±4.5°C.

RESULTS AND DISCUSSION

Root setting

The percentage of seeds that absorbed water within the first 3 days was 100, 100, 100, 82, 82, 70, 8, 82, 70% for Treatments 1 to 9, respectively. The expected rates of root setting for time in each treatment are shown in Figure 2. Rate of root setting was highest in treatment 1 (Cut-Hilum), with most seeds producing roots (that is, produced a root of > 2 mm) within 3 days, and all seeds rooted within 5 days in our experiment. Rate of root setting was also comparatively high in treatment 2 (Cut-Side) and 4 (Scr-Bo), with a cumulative rate of root setting peaking at approximately 9 days. In treatments 3 (Cut-Bot), 6 (Scr-Sul) and 7 (Sul), cumulative rate of root setting gradually increased, while root setting was very limited in the seeds subjected to treatments 5 (Bo), 8 (Scr) and 9 (Cont). Significant differences in germination percentages among the treatments were detected in Log-rank test ($p < 0.01$).

Abari et al. (2012) and Azazi et al. (2013), reported a slightly different results; treatments 5 (Bo) and 8 (Scr) were associated with high germination percentages (around 75%) in their studies. One possible reason for this disparity is because the volume of boiling water used in treatments 4 (Scr-Bo) and 5 (Bo) in this study was small as the treatments were conducted in Petri dishes. It is thus possible that the boiling water may have cooled very quickly, affecting germination efficiency. To comprehensively test the various effects of boiling water on seed germination, conditions such as soaking time and different water temperatures should be examined (Rehman et al., 1999). However, complicated pretreatment conditions are not ideal for local practitioners, such as farmers. In treatment 8 (Scr), it is possible that the low rate of water absorption may have been because we only lightly scratched the seed coat. Success rate of treatment 8 may depend on the technique and/or device used by the practitioner. Given the difficulties and time consuming nature of using sandpaper, Abari et al. (2012) concluded that this method is not recommend for promoting germination in Acacia seeds. On the other hand, the results of treatment 4 (Scr-Bo) indicate that combined use of mechanical scarification and treatment with boiling water may promote germination.

In this study, we found that roots always grow from the point where the seed coat was removed in treatment 1 (that is, the hilum). The seed coat is known to retain elasticity, even if the seed absorbs water, and our results suggest that root setting may sometimes be prevented and/or take time to break through the seed coat (treatment 1 (Cut-Hilum) vs. 2 (Cut-Side), 3 (Cut-Bot)). Indeed, this ability to penetrate the seed coat is considered to be the main cause of variation in rate and duration of germination. Consequently, by identifying the point of root protrusion in treatment 1, the findings of this study will be useful in maximizing germination of A. tortilis seed. All rooted seeds were planted in composted soil and we confirmed that cotyledons opened within 3 days after sowing. Therefore, the efficiency of root setting was directly linked to seedling establishment (Table 1).
Germination percentages

In this study, 100 seeds from each of the seven treatments were sown in sand. Of these, 94, 32, 5, 86, 85, 21 and 6% germinated within 28 days in treatments 1 and 4 to 9, respectively. Significant differences in germination percentage were observed between treatments 1 and 4, 5, 8, 9; 4 and 5; 6 and 4, 5, 8, 9: 7 and 4, 5, 8, 9; 8 and 5, 9 (Ryan’s method, p < 0.01). Although the germination percentages for treatments 4, 5 and 8 were lower than those of the previous studies (Abari et al., 2012; Azazi et al., 2013), this may be attributed to differences in the way mechanical scarification was performed and how boiling water was applied. On the other hand, compared to the previous studies, germination percentages in treatments 6 (Scr-Sul) and 7 (Sul) were similar. Because treatments 6 and 7 in our experiments showed similar germination percentages, mechanical scarification is not considered to be useful in combination with sulphuric acid treatment. In above root setting experiments, 54 and 50% of the seeds in treatments 6 and 7 produced roots within 14 days, respectively. However, 86 and 85% of the seeds in treatments 6 and 7 in this experiment germinated. These results suggest that seeds that have been exposed to chemical treatment take longer to germinate, but that final germination levels in such seeds are not necessarily always low. Nevertheless, germination levels of the seeds in treatment 1 (Cut-Hilum) were higher than the levels of seeds in treatments 6 and 7. Thus, while it may be time consuming to remove seed coat (approximately five seconds/seed), the levels of germination that can be obtained using our method (that is, treatment 1; Cut-Hilum)
Table 1. The summary results of root setting experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage of absorbed water within 3 days (%)</th>
<th>Percentage of root setting within 14 days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 1 (Cut-Hilum)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Treatment 2 (Cut-side)</td>
<td>100</td>
<td>72</td>
</tr>
<tr>
<td>Treatment 3 (Cut-Bo)</td>
<td>100</td>
<td>56</td>
</tr>
<tr>
<td>Treatment 4 (Cut-Bo)</td>
<td>82</td>
<td>70</td>
</tr>
<tr>
<td>Treatment 5 (Cut-Bo)</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Treatment 6 (Cut-Sul)</td>
<td>82</td>
<td>54</td>
</tr>
<tr>
<td>Treatment 7 (Cut-Sul)</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>Treatment 8 (Scr)</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Treatment 9 (Con)</td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2. The results of germination in soil

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 1 (Cut-Hilum)</td>
<td>94</td>
</tr>
<tr>
<td>Treatment 4 (Scr-Bo)</td>
<td>32</td>
</tr>
<tr>
<td>Treatment 5 (Bo)</td>
<td>5</td>
</tr>
<tr>
<td>Treatment 6 (Scr-Sul)</td>
<td>86</td>
</tr>
<tr>
<td>Treatment 7 (Sul)</td>
<td>85</td>
</tr>
<tr>
<td>Treatment 8 (Scr)</td>
<td>21</td>
</tr>
<tr>
<td>Treatment 9 (Con)</td>
<td>6</td>
</tr>
</tbody>
</table>

are higher than those that can be achieved with sandpaper. Our new method should therefore be considered for seed pre-treatment in *A. tortilis* cultivation (Table 2).

**Conclusion**

We consider that the most efficient strategy for promoting germination in *A. tortilis* seeds is as follows: 1) Remove the upper seed coat, 2) soak the seed in water until root protrusion, and 3) transfer the rooted seed to a pot or planter. Using this method, it may be possible to minimise the number of pots required and increase the probability of successfully producing seedlings by more than 90%. In addition, the amount of time required to obtain seedlings for transplanting to pots will be approximately one week.

**Conflict of Interests**

The author(s) have not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

We wish to thank Dr K. Kato and Mr S. Yamaguchi for their kind support during the experiments. This work was carried out as part of KEFRI/JICA project (Development of Drought Tolerant Trees for Adaptation to Climate Change in Drylands of Kenya).

**REFERENCES**


Full Length Research Paper

Chrysodeixis includens (Lepidoptera: Noctuidae) on soybean treated with resistance inducers

Paulo Vinicius de Souza, Bruna Ribeiro Machado, Marcelo Mueller de Freitas, Fernanda Correa, André Cirilo de Sousa Almeida and Flávio Gonçalves de Jesus*

Instituto Federal Goiano, Rod. Prof. Geraldo Silva Nascimento, Km 2,5 CEP. 75790-000, Urutaí, Goiás, Brasil.

Received 9 September, 2014; Accepted 17 November, 2014

Plant resistance levels can be boosted to enable better pest management. The objective of this study was to evaluate resistance interaction between four soybean genotypes and three inducers resistant to integrated management of Chrysodeixis includens. The assays were performed in the entomology laboratory. The soybean genotypes were seeded and plants were treated with calcium and magnesium silicate on soil and sodium silicate and Acibenzolar-S-methyl (ASM) spray on leaves. The weight, longevity and viability of the larvae, the duration and viability of the pre-pupal stage; the weight, longevity and viability of the pupae and the duration and viability of the egg to adult stage of C. includens were observed using plants leaves. The resistant cultivars IAC 100 and IAC 17 associated with the calcium and magnesium silicate inducers and sodium silicate lengthened larval stage and induced high mortality in the C. includens adults.

Key words: Insect, pest management, plant resistance to insects.

INTRODUCTION

Anticarsia gemmatalis Hübner and Chrysodeixis includens Walker (Lepidoptera: Noctuidae) are major pests in soybean in Brazil (Bueno et al., 2012; Mourão et al., 2014). The last species has been found to occur more frequently and in high populations in this crop (Bueno et al., 2012; Silva et al., 2012). These larvae feed on the leaves, leaving behind only the veins, which drastically reduces the leaf area, transpiration and photosynthesis of soybean plants and, consequently, the yield of this crop (Fortunato et al., 2004; Owen et al., 2013).

Chemical insecticides cause undesirable effects (Sosa-Gómez and Silva, 2010) which increases the reason to find effective alternative methods for pest control (Zanuncio et al., 2010; Fagundes et al., 2013; Souza et al., 2014). The alternative methods includes induction of plant resistance using silicon (Almeida et al., 2008, 2009; Lemes et al., 2011), plant resistance to insect, biological control or interaction between the various control methods (McPherson and Buss, 2007; Souza et al., 2012; Zanuncio et al., 2012; Jesus et al., 2014).

Inducers like silicon and Acibenzolar-S-methyl (ASM) can increase the resistance levels without in any way altering the plant genome (Lemes et al., 2011; Peixoto et al., 2011). The silicon increases the plant resistance stimulating growth and protecting plant against biotic and abiotic’s stresses; rising silica mechanical barrier on the
leaf tissues and trichomes as well producing phenolic defensive compounds (Lemes et al., 2011; Cruz et al., 2012). The ASM may activate the genes that encode resistance against biotic's agents (Goussain et al., 2002; Costa et al., 2007).

Resistance inducers have revealed promising results to other insect species on different crops. Silicon application reduce the number of nymphs on peanut plants (Dalastra et al., 2011) and reproduction and biological parameters of Schizaphis graminum Rondani (Hemiptera: Aphididae) in sorghum and wheat (Costa et al., 2007; Pereira et al., 2012).

Rhopalosiphum maidis Fitch (Hemiptera: Aphididae) showed a lower degree of preference for the corn plants treated with silicon (Moraes et al., 2005) and this treatment induced the non-preference mechanism to Aphis gossypii Glover (Hemiptera: Aphididae) in cotton plants (Alcantra et al., 2010). The calcium and magnesium silicate added, increased the number of trichomes in the resistant cultivars IAC 100 and IAC 17 and induced non-preference by Euschistus heros Fabricius (Hemiptera: Pentatomidae) to these cultivars (Souza et al., 2014).

Thus the objective of this work was to evaluate the interactions effects between resistance inducers and soybean cultivars on the biology of the Soybean Looper Chrysodeixis includens.

MATERIALS AND METHODS

The experiment was conducted in the Entomology Laboratory from Instituto Federal Goiano – Campus Uratii, Goiás, Brasil with four soybean cultivars and three resistant inducers from September 2012 to July 2013. C. includens adults were collected and maintained in plastic cages (21.5 cm high and 14.5 cm diameter) with the BRS valiosa soybean cultivar for oviposition and the eggs of this insect were collected.

The C. includens ovipositions were daily removed and placed in plastic cups (16.5 × 5 cm) until caterpillar ecdysis occurred and these were reared on an artificial diet (Greene et al., 1976). The C. includens adults which emerged were placed in plastic cages (21.5 cm high and 14.5 cm to diameter) with 10% honey solution soaked in a cotton wad placed in PET type soft drink cover. C. includens rearing was maintained in an acclimatized room (25±2°C, 70±10% RH and 14 h photoperiod).

IAC 100 and IAC 17 (resistant), BRS Conquista (moderate resistant) and BRS Jatai (susceptible) seeds were treated with Captan fungicide and seeded in plastics pots (5 L) on an organic substrate (29) to obtain the leaves used in the experiments.

The treatments included spraying the plants with 0.3% ASM solution (T1) until draining; application of calcium and magnesium silicate on the soil (T2); 1% sodium silicate (T3); and distilled water (T4) 15 days after plant emergence. The leaves were collected after 30 days and transported to the laboratory to study the biology of insect pest.

Chrysodeixis includens on soybean cultivars treated with resistant inducers

This experiment was conducted using a completely randomized design in a factorial scheme (4 cultivars × 3 inducers + untreated) with 16 treatments and 10 replications. One Petri dish (6 cm diameter lined with moistened filter paper) constituted a replication. The apical leaves of each soybean cultivar per treatment were placed with one newly hatched caterpillar and the biological cycle of C. includens was followed. The leaves of each of the cultivars were daily changed.

The biological parameters evaluated included, a) larva stage: larval period and weight of the ten-day-old caterpillars; b) pupa stage: pupal period and weight of the 24-hour-old pupa and c) egg-to-adult period, longevity and the total cycle of C. includens.

Statistical analyses

Values from biological parameters were submitted to variance analysis (ANOVA) as well as to the F-test. Means values were compared with the Tukey test at 5% probability employing the Sisvar software (Ferreira, 2011).

RESULTS AND DISCUSSION

The larval, pupal periods, weights, adults longevity and development period to egg and to adult of C. includens differed among the soybean cultivars tested. The resistance inducers were observed to affect the pupal period, weight and development period of this insect (Table 1).

C. includens larval and pupal weights were lower (98.10 and 55.70 mg, respectively) when feed to IAC 100 cultivar, and also larval period was longer (15 days). This genotype is considered highly resistant and less damaged by Spodoptera eridania Smith (Lepidoptera: Noctuidae) and the stink bug E. heros (Souza et al., 2012; Souza et al., 2014).

The negative impact of the IAC 100 cultivar on C. includens shows the indirect defense which may be due to increase production of the secondary compounds induced by the herbivory performed by the lepidopteran larvae (Piubelli et al., 2003; Li et al., 2004; Piubelli et al., 2005). These inducer compounds include the flavonoids, mainly rutin and the genistein, which can reduce the degree of feeding by the C. includens (Hoffmann-Campo et al., 2001).

The development period of C. includens was longer with the resistance inducer ASM (26.94 days), than with the other treatments, Ca+Mg silicate (19.75) and Na silicate (20.19 days). These results in the lengthened duration of the life cycle of Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) on the cucumber plants treated with the ASM (Correa et al., 2005).

The larval period (14.87 days) of C. includens was longer on the BRS Jatai (susceptible) with the resistance inducer ASM than that observed in the IAC 17 and BRS Conquista (Table 2). This demonstrates the resistance induction by the ASM in plants (Goussain et al., 2002; Costa et al., 2007).

Significant interaction was observed between inducers and IAC 17 cultivar. The calcium and magnesium silicates lengthened the larval stage of C. includens; the phase was completed in 15.50 days and 2.6 days
The effect of the ASM inducer ranged amongst the cultivars. *C. includens* larvae had less weight when fed on the BRS Jataí (119.8 mg), showing the resistance being induced in the susceptible cultivar (Table 2). On the other hand, the larvae weight was higher on the BRS Conquista (moderate resistance) (181.0 mg). This difference regarding larval weight shows that the ASM can induce resistance against the biotic agents, as longer than that seen in the control, a fact that could be explained by the higher lignin content of the soybean genotype (Moraes et al., 2009) or by the lower degree of feeding by the *S. frugiperda* caterpillars on corn due to the mechanical barrier caused by the silicon accumulation (Goussain et al., 2002).

Table 1. Larval period (days), larval weight (mg), pupal period (days), pupal weight (mg), adult longevity (days) and egg-to-adult period (days) of *Chrysodeixis includens* (Lepidoptera: Noctuidae) in soybean cultivars treated with the resistance inducers ASM, Ca+Mg silicate, Na silicate and untreated (25°C, 70% RH and 14 h photoperiod).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BRS Jataí</td>
<td>13.62a</td>
<td>144.00b</td>
<td>6.33a</td>
<td>126.90b</td>
<td>3.75a</td>
<td>27.12a</td>
</tr>
<tr>
<td>BRS Conquista</td>
<td>13.47b</td>
<td>166.60a</td>
<td>6.62a</td>
<td>149.20a</td>
<td>4.06a</td>
<td>27.00b</td>
</tr>
<tr>
<td>IAC 17</td>
<td>13.84ab</td>
<td>154.30ab</td>
<td>5.96a</td>
<td>141.90a</td>
<td>3.00b</td>
<td>26.19a</td>
</tr>
<tr>
<td>IAC 100</td>
<td>15.00a</td>
<td>98.10c</td>
<td>3.92b</td>
<td>55.70c</td>
<td>0.44c</td>
<td>6.69b</td>
</tr>
<tr>
<td>F (C)</td>
<td>3.28*</td>
<td>24.41**</td>
<td>17.12**</td>
<td>157.25**</td>
<td>52.61**</td>
<td>107.45**</td>
</tr>
</tbody>
</table>

**Means followed by the same lower-case letter do not differ significantly according to Tukey’s test at 0.05 probability.**

Table 2. Larval period (days) and larval weight (mg) of *Chrysodeixis includens* (Lepidoptera: Noctuidae) in soybean cultivars treated with the resistance inducers ASM, Ca+Mg silicate, Na silicate and untreated (25°C, 70% RH and 14 h photoperiod).

<table>
<thead>
<tr>
<th>Cultivars (C)</th>
<th>ASM</th>
<th>Ca + Mg</th>
<th>Na</th>
<th>Untreated</th>
<th>F (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval period (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRS Jataí</td>
<td>14.87a</td>
<td>13.00b</td>
<td>13.12a</td>
<td>13.50ab</td>
<td>1.26ns</td>
</tr>
<tr>
<td>BRS Conquista</td>
<td>12.12ab</td>
<td>13.50ab</td>
<td>14.12ab</td>
<td>14.12ab</td>
<td>1.51ns</td>
</tr>
<tr>
<td>IAC 17</td>
<td>12.50ab</td>
<td>15.50ab</td>
<td>14.50ab</td>
<td>12.87ab</td>
<td>3.36*</td>
</tr>
<tr>
<td>IAC 100</td>
<td>14.00ab</td>
<td>16.00a</td>
<td>14.25ab</td>
<td>15.75ab</td>
<td>1.77ns</td>
</tr>
<tr>
<td>F (I)</td>
<td>2.82*</td>
<td>3.69*</td>
<td>0.62ns</td>
<td>2.60*</td>
<td>–</td>
</tr>
</tbody>
</table>

| Larval weight (mg) |
| BRS Jataí    | 119.80 a BC | 169.00 a A | 147.50 ab A | 139.80 ab AB | 2.82* |
| BRS Conquista| 181.00 a A  | 152.70 a A | 165.30 a A  | 167.60 a A  | 0.91ns |
| IAC 17       | 172.90 a AB | 134.40 a A | 145.10 a A  | 164.80 a A  | 2.13ns |
| IAC 100      | 132.90 a BC | 75.20 b B  | 75.70 b B   | 108.80 ab B | 5.35** |
| F (I)        | 6.09** | 11.44**  | 10.66**  | 5.11**   | –     |

**Means followed by the same lower-case letter do not differ significantly according to Tukey’s test at 0.05 probability.**

**Significant at 1% probability.**

**Significant at 5% probability.**

**Not significant.**
reported for *S. frugiperda* on corn and *S. graminum* on wheat (Goussain et al., 2002; Costa et al., 2007).

The weight of the *C. includens* larvae was lower on the IAC 100 cultivar with the use of the calcium, magnesium and sodium resistance inducers (75.2 and 75.7 mg). The lower weight of this caterpillar with the silicon inducers can be explained by the Si deposition in the leaf cell walls, forming a mechanical barrier, thus increasing the hardness of the leaf tissues and the wearing out of the caterpillar mandibles, thereby reducing the degree of feeding.

The cultivars showed significant interactions without the use of the resistance inducers (untreated). The *C. includens* caterpillars were heavier (167.6 mg) on the BRS Conquista cultivar with the use of the IAC 100 (108.8 mg). This is similar to the resistance recorded in the IAC 100 cultivar to insects such as *C. includens* and stink bugs (McPherson and Buss, 2007; Souza et al., 2014).

The pupal period of *C. includens* was shorter on the IAC 17 treated with the calcium and magnesium silicate resistance inducer (7.0 days) than with the treatment with sodium silicate (4.50 days) (Table 3). This may be related to the length of the larval stage because each one depends on the earlier one to obtain, synthesize and accumulate the nutritional substances (Fugi et al., 2005). The resistance inducer, sodium silicate, increased the pupal period of *C. includens* on the BRS Conquista (7.0 days) compared with those on the IAC 100 and IAC 17 (4.5 and 1.5 days), respectively. The shorter pupal period of *C. includens* on this latter cultivar showed the antibiosis type of resistance, as observed with the cultivars with all the inducers. Sodium silicate and the treatment without using the inducer (control) showed lower values, with 1.50 days for the pupal stage of this insect compared with the 7.33 days with the inducer ASM, prolonging the *C. includens* pupal stage.

The ASM inducer increased the pupal weight (163.5 mg) of the *C. includens* on the IAC 17 to a higher degree than in the other treatments, more than the IAC 100 (86.90 mg) treated with the calcium and magnesium silicate and the BRS Jatai (115.8mg) (Table 3). This may be related to the increased resistance of the susceptible genotype with the resistance inducer (Costa et al., 2007; Souza et al., 2014).

The *C. includens* larval mortality reached 100% with the use of the inducer sodium silicate; however, in the control it prevented the pupal weight from being recorded with the IAC 100 genotype. Such mortality revealed by the resistant cultivar IAC 100 is explained by the flavonoid rutin and the genistein isoflavones linked to the anti-nutritional effect and injury to cells of the digestive tract as shown for *A. gemmatalis* fed on a diet containing these metabolites (Hoffmann-Campo et al., 2001; Salvador et al., 2010).

The increased longevity of *C. includens* adults on the BRS Conquista without the use of the resistance inducers shows a lower impact on the IAC 17 treated with the sodium silicate on the adults of this insect (Table 4). The mortality of the insects on the IAC 100 cultivar with the use of the calcium and magnesium silicate and sodium silicate inducers and in the control disallowed the evaluation of the longevity of this insect and thus corroborates the beneficial effect of the interaction of the resistant plants with the inducers to control the spread of *E. heros* on soybean (Souza et al., 2014).

---

**Table 3. Pupal period (days) and pupal weight (mg) of *Chrysodeixis includens* (Lepidoptera: Noctuidae) in soybean cultivars treated with the resistance inducers ASM, Ca+Mg silicate, Na silicate and untreated (25ºC, 70% RH and 14h photoperiod).**

<table>
<thead>
<tr>
<th>Cultivars (C)</th>
<th>ASM</th>
<th>Ca + Mg</th>
<th>Na</th>
<th>Untreated</th>
<th>F (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pupal period (days)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRS Jatai</td>
<td>6.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>BRS Conquista</td>
<td>6.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.55&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>IAC 17</td>
<td>6.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.61*</td>
</tr>
<tr>
<td>IAC 100</td>
<td>7.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>24.11**</td>
</tr>
<tr>
<td><strong>Pupal weight (mg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRS Jatai</td>
<td>115.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>136.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123.40&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>132.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.79&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>BRS Conquista</td>
<td>143.80&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>140.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>146.80&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>165.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.63&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>IAC 17</td>
<td>163.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>145.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>128.60&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>130.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.44**</td>
</tr>
<tr>
<td>IAC 100</td>
<td>136.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>96.84**</td>
</tr>
<tr>
<td><strong>F (I)</strong></td>
<td>8.26**</td>
<td>15.79**</td>
<td>96.67**</td>
<td>114.51**</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>1</sup>Means followed by the same lower-case letter do not differ significantly according to Tukey's test at 0.05 probability. **Significant at 1% probability. *Significant at 5% probability. #Not significant.
The lengthened time for the total cycle of *C. includens* on the IAC 17 cultivar with the calcium and magnesium silicate (28.50 days) than in the control (25.25 days) indicates an adverse effect on the biology of this insect, thus characterizing the antibiotic (Paixão et al., 2013). This may be related to the protective layer formed by the accumulation of silicon on the epidermal cells, which inhibits feeding, thus impacting the life cycle of this insect (Savant et al., 1997; Datnoff et al., 1991).

The IAC 100 cultivar treated with the resistance inducers calcium and magnesium silicate and sodium silicate as well as without them (untreated) increased the length of the larval stage of *C. includens*. This indicates that this effect is caused by the anti-nutritional compounds and induction of the secondary defense metabolites in these plants. The ASM did not prevent this insect from completing its cycle on this genotype.

### Conclusion

The resistant cultivars IAC 100 and IAC 17 associated with the calcium and magnesium silicate inducers and sodium silicate lengthened the larval stage and induced high mortality in the *C. includens* adults.

### Conflict of Interest

The author(s) have not declared any conflict of interests.

### REFERENCES


Correa RSB, Moraes JC, Auad AM, Carvalho GS (2005). Silicon and nutritional com


Full Length Research Paper

**Novel carrier system for enhancing oral delivery of metformin**


1Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria.
2Drug Delivery Research Unit, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.
3Department of Pharmaceutical Technology and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.

Received 10 June, 2014; Accepted 2 December, 2014

This study was designed to evaluate the potential of PEGylated lipospheres as carriers for improved oral delivery of metformin hydrochloride. Lipospheres were prepared by melt-emulsification method using Phospholipon® 90H in beeswax (30%w/w) as the lipid matrix containing increasing quantities of PEG 4000 and characterized. The *in vitro* and *in vivo* release of the formulations was evaluated. Results show that the particle size and encapsulation efficiency ranged from 33.18±1.75 - 83.23±6.05 μm and 85 to 93%, respectively. Drug release showed a biphasic pattern and was found to follow the Higuchi square root model. Metformin hydrochloride-loaded lipospheres lowered basal blood glucose levels by 60% and sustained antihyperglycemia for over 20 h. This study suggests that encapsulation of metformin hydrochloride into PEGylated lipospheres could reduce its dosing frequency and the associated side effects resulting from high doses of metformin hydrochloride as seen in conventional tablet formulations.

**Key words:** PEGylated, lipospheres, metformin hydrochloride, anti-diabetic.

**INTRODUCTION**

The design of an oral controlled drug delivery system should primarily be aimed at achieving more predictable and increased bioavailability of drugs as there are several physiological difficulties, which include restraining/localizing the drug delivery system within the regions of the gastrointestinal tract and the high variable nature of gastric emptying process (Prajapati et al., 2008). Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose concentration caused by insulin deficiency, often combined with insulin resistance (Philip et al., 2009). The effective control of blood glucose is the key in preventing or reversing diabetic complications and improving the quality of life for both type I and type II diabetic patients. Although different

*Corresponding author. E-mail: mo_adedokun@yahoo.com. Tel:+234 (0) 8034396937.

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License*
types of oral hypoglycemic agents are commonly employed along with insulin for the treatment of diabetes mellitus, none offers complete glycemic control (Pradeep, et al., 2010).

Metformin HCl is an effective antidiabetic that requires controlled release owing to its short biological half-life (1.5-4 h) (Stepensky et al., 2001; Momoh et al., 2011). It is used as monotherapy as well as an adjunct to diet in the management of type II diabetes in patients whose hyperglycemia cannot be controlled by diet alone. Quite frequently, it causes gastrointestinal problems such as nausea, stomach pain, bloating, and diarrhea. Metformin hydrochloride is absorbed from the upper intestine within 6 h of administration, so repeated administration is required to maintain effective plasma concentration (Momoh et al., 2013).

Lipospheres were first reported as a particulate dispersion of solid spherical particles between 0.2-100 μm in diameter consisting of solid hydrophobic fat core such as triglycerides or fatty acids derivatives, stabilized by monolayer of phospholipids (Attama et al., 2008, 2009). The internal core contains the drug dissolved or dispersed in solid fat matrix. Lipospheres drug delivery system is an emerging carrier for both hydrophilic and hydrophobic drugs. This carrier system has several advantages over other delivery systems in terms of physical stability, low cost of ingredients, ease of preparation, and scale-up, high dispensability in aqueous medium, high entrapment efficiency, and extended release of entrapped drug (Attama et al., 2009). The novelty of the work lies on the use of beeswax and Phospholipon® 90H (a homo and hetero lipid) in combination with polyvinyl alcohol to improve and enhanced the oral delivery of metformin entrapped lipospheres prepared by fusion method. Recently, lipid-based formulations have been used to control and enhance the release of some drug molecules (Attama et al., 2009; Attama and Nkemnele, 2005). Fusion method enhances the encapsulation of the drug in the core and improves its delivery capacity when in contact with the wall of the gastrointestinal tract (Attama et al., 2009; Philip et al., 2009). The prepared lipospheres were evaluated for production yield, loading efficiency, morphology, particle size, pH analysis, and in vitro drug release. The anti-diabetic property of the formulations was also evaluated in alloxan-induced rat model.

The objective of this study was to evaluate metformin hydrochloride-loaded lipospheres prepared by fusion method for possible oral delivery of metformin, in order to achieve a controlled release and enhanced bioavailability.

MATERIALS AND METHODS

Materials

The following materials were used: Beeswax, Phospholipon® 90H (Nattermann, Germany), Polyvinylalcohol (Sigma-Aldrich, USA), polyethylene glycol 4000 (Cary Roth, Karlsruhe, Germany), Metformin hydrochloride (Farmex, Pharma Ltd, Ikeja, Lagos State, Nigeria), Monobasic potassium phosphate, Sodium hydroxide and Concentrated hydrochloric (BDH, Poole, England), and Distilled water (Lion Water, University of Nigeria, Nsukka, Nigeria). Other reagents were of analytical grade and used without further purification.

Preparation of lipid matrix

The lipid matrix consisting of 4:1 mixture of beeswax and Phospholipon® 90H (P90H) were prepared by fusion method (Attama et al., 2008). Briefly, 80 g quantity of beeswax and 20 g of P90H were weighed using an electronic balance (Mettler H8, Switzerland) and melted together on a crucible at 75°C over a thermo-regulated shaking water bath (Heto, Denmark) and stirred thoroughly to obtain a homogenous mixture. Thereafter, the lipids were allowed to cool and solidify at room temperature to get a lipid matrix.

Preparation of drug loaded and unloaded lipospheres

The melt homogenization technique was adopted (Attama et al., 2008). In each case, the lipid matrix was melted at 70°C, and the aqueous phase containing PEG-4000 and polyvinyl alcohol (PVA) at the same temperature was added to the molten lipid matrix under gentle stirring with a magnetic stirrer (SR 1 UM 52188, Remi Equip., India), and the mixture was further dispersed with a mixer (Ultra-Turrax, Germany) at 8000 rpm for 5 min to produce the hot primary emulsion. The lipospheres suspension obtained after cooling at room temperature was then lyophilized using a freeze-dryer (Amsco GT3, Germany) in order to get water-free lipospheres. Briefly, lyophilisates of the SLMs are obtained by freezing the formulations at a pressure of 2.7 Pa and temperature of 30°C; sublimation and drying were at 15-25°C and all these operations took 6-12 h. The above procedure was repeated using increasing amount of PEG, (0.5, 1.0, 1.5 and 2.0 g), decreasing amount of lipid matrix; (4.5, 4.0, 3.5 and 3.0 g) and two concentration levels of metformin hydrochloride (250 and 500 mg), to obtain metformin hydrochloride-loaded lipospheres (batches A1 - A4 and B1 - B4). Unloaded lipospheres (without drug) were similarly prepared (C1 - C4). The formulation compositions are shown in Table 1.

Characterization of lipospheres

Particle size analysis and morphological characteristics of lipospheres

The particle size of the lipospheres was determined by computerized image analysis. Approximately, 3.0 mg of the lipospheres from each batch was dispersed in distilled water and smeared on a slide (Marinfield, Germany) using a glass rod. Each of the batches on a slide was mounted and observed under a light photo-microscope (Leica, Germany). With the aid of the software in the photomicroscope, the projected diameters of the particles corresponding to the particle sizes of the lipospheres were determined and the average calculated. The particle morphologies were also observed and photomicrographs taken. Measurement of particle size of the formulated lipospheres was repeated at intervals of 24 h, one week and four weeks after formulation.

pH stability studies of the formulations

With the aid of a pH meter (Digital pH Meter, Labtech), the pH values of the different batches of the lipospheres formulations
including those of the control were measured. Measurements were also carried out at one week intervals for one month post formulation.

**Determination of encapsulation efficiency (EE %)**

The encapsulation efficiency of each formulation was determined. A 6 ml volume each of the reconstituted lipospheres was centrifuged for 60 min at an optimized speed of 3000 rpm in order to obtain two phases (aqueous and lipid phases). A 1 ml volume of the aqueous phase was measured out and diluted 1000-fold using distilled water. The absorbance of the solutions at a wavelength of 278 nm, were taken using a UV-spectrometer and the encapsulation efficiency was calculated using the formula below:

\[
EE\% = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100
\]  

(1)

**Determination of loading capacity (LC)**

LC is expressed as the ratio between the entrapped drug by the lipid and the total quantity of lipids used in the formulation and calculated as follows:

\[
\text{LC} = \frac{\text{Total quantity of drug entrapped by the lipid}}{\text{Total quantity of the lipid in the formulation}} \times 100
\]

(2)

**In vitro drug release studies**

A volume (4.0 ml) of the reconstituted lipospheres from each batch was accurately measured and placed in the donor compartment of a Franz diffusion cell that was separated from the receptor compartment by an artificial membrane (pore size 0.22 µm). The receptor compartment was filled with simulated intestinal fluids (SIF) without pancreatin (pH 7.4) and maintained at a temperature of 37±1°C by means of a thermostatically controlled water bath, with agitation being provided by a magnetic stirring bar at 50 rpm. A 2.0 ml was removed and replaced by an equal volume of the receptor phase at predetermined time intervals. The drug contents were analyzed using a spectrophotometer at a wavelength of 283 nm. The amount of drug released at each time interval was determined with reference to the standard Beer’s plot earlier determined for metformin hydrochloride in SIF.

**Pharmacodynamic study**

**Induction of diabetes**

The animal experiments in this work complied with the regulations of the Committee on Ethics on the Use of Laboratory Animals of the University of Nigeria and were in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC) (EEC, 1986). Rats weighing between 200 - 220 g were purchased from the Department of Biochemistry, University of Nigeria, Nsukka. The rats were all kept in standard and conditioned animal cages and left for one week to acclimatize to the new laboratory environment while being fed with standard laboratory chow diet. Diabetes was induced by intravenous injection of Alloxan dissolved in normal saline through the marginal ear vein at a dose of 120 mg/kg (Builders et al., 2008). After 5-7 days of the Alloxan treatment, rats with frequent urination, loss of weight, and blood glucose levels higher than 220 mg/dL were considered diabetic and selected for the study (Builders et al., 2008). The rats were monitored for persistent blood glucose elevation for 5 days (Sharma et al., 2006). Before testing, animals were fasted overnight with free access to water.

**In vivo antidiabetic study**

Twenty five (25) Wistar rats (either sex) of an average weight of 200.0±20.0 g were used for the evaluation of the pharmacological effects of metformin hydrochloride-loaded lipospheres after oral administration. In each case, the animals were fasted for 24 h prior to oral administration of the formulations. Rats were divided into five groups of five animals each and the formulations were administered orally. Briefly, the first group received Batch A₁ containing metformin hydrochloride-loaded lipospheres at a dose of 7 mg/kg body weight. The second group received Batch B₁ also containing metformin hydrochloride-loaded lipospheres at a dose of 0.7 mg/kg body weight. Whereas the third group received Batch C₁ (zero-drug loaded liposphere formulation), the fourth group received a
commercially available metformin hydrochloride preparation (Mephage®) while the last group received metformin hydrochloride dispersed in distilled water (100 mg p.o). The selected formulations (A1 and B1) used in the in vivo study were based on the results of our preliminary evaluation. Blood samples were taken from the tip of the tail vein at predetermined intervals and blood glucose levels were measured using an Accu-Check Roach, USA). Food and water intake as well as urine output of the animals were measured and monitored in the course of the study (Sharma et al., 2006).

Statistical data analysis

All experiments were performed in replicates (n= 3) using SPSS version 18 for validity of statistical analysis. Results were expressed as mean ± SD and differences between means were considered significant at p< 0.05 using the analysis of variance (ANOVA).

RESULTS

The particle size and the representatives of the morpholo-
Table 3. Physicochemical properties of the lipospheres (Mean±SD, n=3).

<table>
<thead>
<tr>
<th>BC</th>
<th>EE(%)</th>
<th>DL%</th>
<th>YD%</th>
<th>Particles size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>A1</td>
<td>92.46</td>
<td>39.31</td>
<td>88.00</td>
<td>53.11±1.1</td>
</tr>
<tr>
<td>A2</td>
<td>90.14</td>
<td>30.13</td>
<td>79.10</td>
<td>49.01±0.2</td>
</tr>
<tr>
<td>A3</td>
<td>86.60</td>
<td>31.02</td>
<td>86.11</td>
<td>51.19±0.11</td>
</tr>
<tr>
<td>A4</td>
<td>85.50</td>
<td>32.47</td>
<td>88.20</td>
<td>51.87±3.01</td>
</tr>
<tr>
<td>B1</td>
<td>84.93</td>
<td>24.51</td>
<td>90.10</td>
<td>52.12±0.13</td>
</tr>
<tr>
<td>B2</td>
<td>83.19</td>
<td>24.91</td>
<td>79.00</td>
<td>55.11±3.75</td>
</tr>
<tr>
<td>B3</td>
<td>68.70</td>
<td>24.68</td>
<td>74.10</td>
<td>62.08±3.75</td>
</tr>
<tr>
<td>B4</td>
<td>65.22</td>
<td>25.15</td>
<td>80.01</td>
<td>56.01±3.75</td>
</tr>
<tr>
<td>C1</td>
<td>-</td>
<td>-</td>
<td>89.13</td>
<td>54.03±5.65</td>
</tr>
<tr>
<td>C2</td>
<td>-</td>
<td>-</td>
<td>86.21</td>
<td>33.18±1.75</td>
</tr>
<tr>
<td>C3</td>
<td>-</td>
<td>-</td>
<td>88.81</td>
<td>43.23±0.12</td>
</tr>
<tr>
<td>C4</td>
<td>-</td>
<td>-</td>
<td>82.03</td>
<td>52.22±1.85</td>
</tr>
</tbody>
</table>

BC = Batch code; EE = % encapsulation efficiency; DL = % drug loading and % yield value. Batches A1 – A4 and B1 – B4 contain 500 and 250 mg of metformin hydrochloride while batches C1 – C4 contain no drug (0 mg).

Figure 2. Release profile of metformin HCl from the lipospheres (Batch A) in SIF. (A1–A4= batches of the formulation contain 500 mg of metformin hydrochloride).

In vitro drug release studies

Figures 2 and 3 show the in vitro release profiles of different batches of the metformin hydrochloride-loaded lipospheres in SIF. There was an initial release of about 15-20% of drug from the metformin hydrochloride-loaded lipospheres within the first 0.5 to 1 h. Drug release from the formulations was sustained for up to 20 h with only unloaded lipospheres. The pH varied from between 3.5 to 4.6 after one week of preparation to between 4.7 and 5.6 after four weeks of preparation, while the unloaded lipospheres (C1-C4) recorded the highest variation in pH after four weeks of formulation. Results revealed that the EE % decreased as the amount of polymer (PEG-4000) in the lipid matrix increased.

In other words, EE % decreased as the lipid base decreased (Table 3) but increased with increase in the concentration of the drug such that the maximum EE % was 92.58% for lipospheres (batches A) containing 500 mg of metformin hydrochloride, as compared to 84.93% recorded for lipospheres (batches B) containing 250 mg of the drug (Table 3).
Figure 3. Release profile of metformin HCl from the lipospheres (Batch B) in SIF. (B1-B4 = batches of the formulation contain 250 mg of metformin hydrochloride).

Table 4. Release kinetics of the lipospheres.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Higuchi’s plot</th>
<th>Korsmeyer-Peppas plot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r²</td>
<td>r²</td>
</tr>
<tr>
<td>A₁</td>
<td>0.947</td>
<td>0.896</td>
</tr>
<tr>
<td>A₂</td>
<td>0.963</td>
<td>0.880</td>
</tr>
<tr>
<td>A₃</td>
<td>0.963</td>
<td>0.898</td>
</tr>
<tr>
<td>A₄</td>
<td>0.956</td>
<td>0.865</td>
</tr>
<tr>
<td>B₁</td>
<td>0.762</td>
<td>0.966</td>
</tr>
<tr>
<td>B₂</td>
<td>0.672</td>
<td>0.989</td>
</tr>
<tr>
<td>B₃</td>
<td>0.762</td>
<td>0.926</td>
</tr>
<tr>
<td>B₄</td>
<td>0.779</td>
<td>0.920</td>
</tr>
</tbody>
</table>

A₁-A₄ and B₁-B₄ = batches of the formulation contain 500 and 250 mg, respectively of metformin hydrochloride.

50% of the metformin hydrochloride being released after 16 h.

Modeling of in vitro drug release

The release mechanism for metformin hydrochloride-loaded lipospheres is shown in Table 4. The in vitro release profile of metformin hydrochloride from the lipospheres was fitted into the Higuchi equation and the plots showed high linearity ($r^2 > 0.999$).

Pharmacodynamic studies

Figure 4 shows the changes in blood glucose levels after the formulations were administered to diabetic rats. At 7.0 mg/kg body weight, orally delivered metformin hydrochloride-loaded lipospheres lowered basal blood glucose levels in diabetic rats by 60% and sustained hypoglycemia for over 20 h. No reduction in blood glucose levels was observed in rats that received no treatment. Reduction in blood glucose levels was similarly observed in rats that received pure sample of metformin hydrochloride and the commercially available metformin hydrochloride marketed sample (Mephage®). Although, there was sustained antihyperglycemia in all cases, for about 10 h, the hypoglycemic effect was greater in rats that received the PEGylated microscopic lipospheres than those that received the latter (pure sample of metformin hydrochloride and Mephage®). The result here further suggests that the release of metformin hydrochloride from the lipospheres stimulated the release of insulin from the production cell or stimulated the tissue uptake of glucose or both in a controlled manner as was observed in the glucose lowering effect.
Figure 4. Percentage reduction in blood glucose level in diabetes rats after oral administration of metformin hydrochloride lipospheres formulations (A₁, B₁ and C₁ contain 500, 250 and 0 mg of metformin hydrochloride), Mkt = market sample and PD= Pure drug.

DISCUSSION

In this study, a PEGylated microscopic lipospheres delivery system of metformin hydrochloride was developed and evaluated for enhanced oral delivery of metformin hydrochloride. The lipospheres formed were fairly smooth and spherical in shape, and showed some consistency according to the drug incorporated into the formulation (Figure 1). There were no signs of sedimentation after the formulations stood for a period of 8 weeks. There was a slight increase in the particle sizes after four weeks. The increase in the particle size was directly proportional to the amount of drug entrapped in the formulation. This increase may be due to crystallization of the formerly molten matrices or it may be due to aggregation and subsequent growth by Ostwald ripening or sintering (Attama et al., 2008; Attama et al., 2009). The increase in particle size was not significant (p>0.05).

The pH of the different batches of lipospheres was measured at one week intervals up to one month after preparation to ascertain the variation of pH with time, which could be a function of degradation of the drug or lipid component or both. In pharmaceutical formulations, the initial preparation may be stable, but as time goes on, degradation of either the components or the drug or both may set in on storage through generation of unfavorable pH (increase or decrease) or reactive species from the drug.

It is therefore of paramount importance to determine the pH of maximum stability for formulations and utilize the knowledge in designing a suitable formulation for the drug (Sepici, et al., 2004). This will serve as a guide for the formulator on the need of adding a preservative or stabilizer to ensure that a stable product is achieved and maintained throughout the shelf-life of the product. The slight increase in the pH values in all the lipospheres (Table 2) may not be attributable to drug degradation since there was also a rise in the pH of the unloaded lipospheres. Degradation of the lipidic excipients, common to all the formulated lipospheres, to free fatty acids could occur, causing a fall in the pH of the formulation rather than a rise in pH as was observed in the present study, which is consistent with earlier report (Attama and Nkemnele, 2005), suggesting that it is neither the drug nor the excipients that caused the rise in pH. It would, therefore, be reasonable to infer that the rise in pH is due either to a rise in the particle surface pH or the likely interaction of the ions present in the medium with the components of the formulations (Gao, et al., 2004).

The function of the formulated lipospheres is to deliver the active pharmaceutical ingredient (API) for further absorption into the biological system (Attama et al., 2009; Nnamani et al., 2007). This can be expressed or determined by the EE % and LC. Encapsulation efficiency is a function of the amount of drug entrapped in lipid base to the total weight of drug while the loading capacity expresses the ratio between the entrapped drug and the total weight of lipid used as a carrier (Attama et al., 2009; Nnamani et al., 2007).
Results reveal that the EE % decreased as the amount of polymer (PEG-4000) in the lipid matrix increased. In other words, EE % decreased as the lipid base decreased (Table 3) but increased with increase in the concentration of the drug (Table 3). This showed that the polymer (PEG-4000) together with the lipid matrix promoted drug solubilization in a concentration-dependent manner. The lipid matrix accommodated more drugs at higher drug loadings possibly due to the low crystalline nature of the excipients. The variation in the EE % as shown in Table 3 is a clear indication that both the lipid and PEG contents are critical variables in the amount of drug that could be entrapped by the formulated lipospheres.

Loading capacity, however, increased as the amount of polymer (PEG-4000) increased and as the amount of the lipid matrix decreased. Batches A lipospheres recorded greater loading capacity than Batches in B lipospheres (Table 3). The presence of PEG-4000 in the lipospheres batches A1-A4, B1-B4 and C1-C4 was found to be essential in obtaining spherical particles (Figures not shown). The yields of the lipospheres suggested that the processing parameters and the fusion method employed in the preparation of the liposphere did not affect the yield, thus making the process scalable and this could be of commercial interest.

The particle size of lipospheres (C1-C4) containing no API but formulated with 10 % of PEG were slightly larger than those formulated with 30% PEG-4000. The particle size and morphology of metformin hydrochloride - loaded lipospheres are shown in Table 3 and Figures 1, respectively.

Drug release is affected by the nature or design of the delivery system and the medium used in the release study. Factors such as pH are among the most important factors affecting drug release. Other factors such as viscosity and gastro intestinal motility affect the in vitro release of drugs while in vivo release is affected by agitation, viscosity and temperature of the medium, stirring speed of the apparatus used in the release study. Figures 2 and 3 show the in vitro release profiles of different batches of the metformin hydrochloride-loaded lipospheres in SIF. There was an initial release of about 47% and 49% respectively was achieved by the pure sample of metformin hydrochloride dispersed in distilled water and the commercial product (Mephage™). The order of hypoglycemic activity of formulated metformin hydrochloride -loaded lipospheres are batch A1 > batch B1 > PD > MK > batch C1. This shows that batches A1 and B1 possessed greater antihyperglycemic effect than commercially available metformin HCl formulation and pur sample of metformin hydrochloride, an indication that entrapment of metformin hydrochloride into PEGylated lipospheres not only improved its therapeutic effectiveness but also extended its therapeutic action, consistent with a similar reported study (Attama et al., 2009; Nnamani et al., 2007).

Results of the in vivo pharmacodynamic study show that percentage reduction of basal blood glucose levels of 47 and 49% respectively was achieved by the pure sample of metformin hydrochloride dispersed in distilled water and the commercial product (Mephage™). The order of hypoglycemic activity of formulated metformin hydrochloride -loaded lipospheres are batch A1 > batch B1 > PD > MK > batch C1. This shows that batches A1 and B1 possessed greater antihyperglycemic effect than commercially available metformin HCl formulation and pur sample of metformin hydrochloride, an indication that entrapment of metformin hydrochloride into PEGylated lipospheres not only improved its therapeutic effectiveness but also extended its therapeutic action, consistent with a similar reported study (Attama et al., 2009; Nnamani et al., 2007).

Conclusion

In designing a drug delivery device such as those based on polymers or lipids or combinations thereof, the choice as well as the combination ratio of the polymers or lipids or other excipients should be considered based on the desired pattern, route of administration, stability, and other physicochemical considerations. In this research work, metformin hydrochloride-loaded lipospheres were successfully prepared and optimized and evaluated in a diabetic animal model.

Majority of the formulated lipospheres of metformin hydrochloride exhibited better in vivo performance than commercially available MT formulation. In the light of the importance of metformin hydrochloride in the management of type II diabetes mellitus, the formulated lipospheres...
could further be exploited as an alternative dosage form for metformin hydrochloride. In view of the sustained hypoglycemic effect that was observed in metformin hydrochloride-loaded PEGylated lipospheres, the studied formulation would be a necessary intervention in reducing the dosing frequency of metformin hydrochloride with concomitant reduction in the side effects associated with it.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES


Luan X (2006). Key parameters affecting the initial release (burst) and encapsulation efficiency of peptide-containing poly(lactide-co-glycolide) microparticles. Int. J. Pharm. 324:168-175.


Differential responses of growth, chlorophyll content, lipid peroxidation and accumulation of compatible solutes to salt stress in peanut (Arachis hypogaea L.) cultivars

Meguekam Liliane Tekam1, Taffouo Victor Désiré2*, Grigore Marius-Nicusor3, Zamfirache Magdalena Maria3, Youmbi Emmanuel4 and Amougou Akoa4

1Department of Biological Sciences, Higher’s Teacher Training College, University of Yaoundé I, 47 Yaoundé - Cameroon.
2Department of Botany, Faculty of Sciences, University of Douala, 24157 Douala - Cameroon.
3Department of Plant Biology, Faculty of Sciences, University of Yaoundé I, 812 Yaoundé - Cameroon.
4Faculty of Biology, University of Alexandru Ioan Cuza de Iaşi, Street, Carol I, nr., 11/ 700506, Romania.

Received 15 October, 2014; Accepted 24 November, 2014

The present study was aimed to compare differential responses of growth, chlorophyll content, lipid peroxidation and accumulation of compatible solutes in peanut (Arachis hypogaea L.) cultivars: Fleur 11 (salt-tolerant), Mbiah and PC 79-79 (moderately-tolerant) and Vanda (salt-sensitive) at the vegetative growth stage, under greenhouse conditions, in the presence of 0, 50, 100 or 200 mM NaCl. The root dry weight (RDW) and shoot dry weight (SDW) of cv. Vanda decreased significantly (p< 0.05) in salt-treated plants than those of cvs. Fleur 11, Mbiah and PC 79-79. The SDW reduction was notably noted at 100 mM NaCl in cvs. Mbiah and PC 79-79, while cv. Fleur 11 showed significantly (P<0.05) decrease in salt-treated plants only at 200 mM NaCl but had higher SDW accumulation than others. The leaf chlorophyll content increased in cvs. Fleur 11 and PC 79-79 and decreased in cv. Vanda with increasing NaCl levels. Proline (PRO) and glycine betaine (GB) contents significantly (p<0.05) increase, for all cultivars, in the stressed plants with the highest quantity in Fleur 11 and the lowest in Vanda. Malondialdehyde levels increased under salt stress in the leaves of cv. Vanda but decreased in cvsMbiah, PC 79-79 and Fleur 11 at 100 and 200 mM, respectively. Total phenolic content increased significantly (p < 0.05) at 200 mM NaCl in the leaves of cv. Vanda than others, while cv. Fleur 11 showed the lowest increase. The salt-tolerant cv. Fleur 11 exhibits a better protection mechanism against oxidative damage caused by salt stress maintaining a higher accumulation of PRO and GB than others. Higher PRO and GB accumulation in the leaves may be regarded as potential biochemical indicator for earlier selection of salt tolerant peanut and targets for improvement through transgenic approaches.

Keywords: Arachis hypogaea, glycine betaine, growth, lipid peroxidation, proline, salt stress

INTRODUCTION

Salinity stress is the most limiting factor in agricultural productivity in arid and semi-arid regions of the world (Horie and Schroeder, 2004). In saline environment excessive sodium affects plant growth of many sensitive species which comprised most of crops (Hossein and Fatemeh, 2012; Rameeh et al., 2012). The morphological,
physiological and biochemical attributes of plants is altered by salinity thus limiting its growth and development (Mudgal et al., 2010). Low external water potential, ion toxicity and interference with the uptake of nutrients are a range of mechanisms that inhibit plant growth due to salinity (Munns et al., 1995). The responses of plants to high soil salinity and the mechanisms of salt tolerance have been largely discussed (Ruan et al., 2010; Grigore et al., 2011). Compartmentation of ions in vacuoles and accumulation of compatible solutes in the cytoplasm are commonly proposing mechanisms of salt tolerance species (Munns, 2002). The basic mechanisms of salt tolerance in halophytes seem to be mostly dependent in their capacity to sequester toxic ions (Na+, Cl−) in the vacuoles and to accumulate compatible osmotica in the cytoplasm (Le Rudulier, 2005; Grigore et al., 2011). The compatible solutes accumulation that are mostly seen in plants are proline (PRO) and glycine betaine (GB), but other osmolytes can be stored at high concentrations in some species (Girija et al., 2002). An increase in salinity increases PRO as an adaptative change in metabolism pattern (Mudgal et al., 2010). Thylakoid and plasma membrane integrity are protected by GB after exposure to saline solutions (Rhodes et al., 1987).

Peanut salt-tolerant cultivars accumulate the highest quantity of GB while moderately-tolerant cultivar stored intermediate amount and sensitive low quantity (Girija et al., 2002). Thus, the accumulation of osmo-protectants in tissues of plants growing in arid or semi-arid lands, may exhibit more tolerance to salt stress (Munns, 2002). Salt stress can lead to stomatal closure, which reduces CO2 availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn could increase the generation of Reactive Oxygen Species (ROS) and induce oxidative stress (Parvaiz and Satyawati, 2008). Plants have developed a series of enzymatic and non-enzymatic detoxification systems to counteract ROS, and protect cells from oxidative damage (Sairam and Tyagi, 2004). The assessment of cell membrane stability is an appropriate technique to screen plants under saline condition (Munns et al., 2006). Salt stress increased lipid peroxidation or induced oxidative stress in plant tissues (Hernandez et al., 1993). Malondialdehyde (MDA) has been known as the end product of peroxidation of membrane lipids (Sajedi et al., 2011). Increase in the level of MDA, produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage (Azad et al., 2012). The salt stress was able to induce excessive generation of MDA in the root and leaf of maize seedlings (Azad et al., 2012). Phenolic compounds are a large group of secondary metabolites, which can play a role in any interaction that a plant can have with its environment (Waterman and Mole, 1994). These compounds have been implicated to stress resistance against biotic and abiotic factors (Bergmann et al., 1994). Total phenolic (TP) accumulation could be a cellular adaptive mechanism for scavenging oxygen free radicals during stress (Mohamed and Aly, 2008). The increased synthesis of PRO, TP and the antioxidant activity in dill seedlings exhibited a protective mechanism against the cellular structures from oxidative damage (Zahra et al., 2012).

Grain legumes provide large amounts of high quality proteins which contain relatively more of the essential amino acids not supplied by cereals in which the content of lysine and tryptophan are relatively small (Kay, 1979). Peanuts are essential sources of fat (34 to 54%) (Nyabyenda, 2005). Legumes intervene in crop rotation systems and participate in biological nitrogen fixation (Delgado et al., 1994). The selection of tolerant cultivars can be done efficiently in cultivated saline environments, and thus salt tolerance mechanisms potential can be identified within plant species which is becoming an increasing research priority in many countries. It is important to make a call to the ecophysiological approach which can constitute an attenuation of the effect of the soil’s salinity on the cultivated plant performances (Mekhaldi et al., 2008). This would lead to the search of tolerant species or varieties of plant thus imposing a mastery of the knowledge of mechanisms to their adaptation to salinity.

The present study was aimed to compare differential responses of growth, physiological and biochemical characteristics in peanut cultivars differing in salt tolerance at the first vegetative growth stage and determine biochemical indicators which could serve as early selection criteria for tolerance of salt in peanut.

**MATERIALS AND METHODS**

**Plant material, growth and stress conditions**

Experiments were performed using seeds of four peanut (Arachis hypogaea L.) cultivars differing in salt tolerance; cv. Fleur 11 (salt-tolerant), cvs. Mbiah and PC 79–79 (moderately-tolerant) and cv. Vanda (salt-sensitive). Mbiah and Vanda were provided by the breeding program of the Agronomic Institute for Research and Development of Cameroon. Fleur 11 and Pc 79–79 were obtained from Senegalese Institute of Agronomic Research. Germination trials were conducted in 9 cm sterile Petri dishes lined with Whatman No.1 filter papers and moistened with 10 mL of distilled water. After seed surface sterilization with 70% (v/v) ethanol solution for 15 min, followed by rinsing with distilled water, seeds were sowed in Petri dishes and placed in seed germinator at 26°C for 5 d. After germination, the plants were sown in pots with sterilized sand in a greenhouse (26/23°C light/dark and 51 to 61%
hygrometry), located at the Alexandru Ioan Cuza de Iaşi University, Romania, from June to September, 2011 and March to July, 2012. The pots were arranged in a complete randomized design with five replicates. One plant was grown in the middle of each pot. Each cultivar had 20 pots divided into four groups. Each group, with five replicates, were fertilized every two days with the nutrient solution (Bolder et al., 1983) containing 49.2% CaCO$_3$, 13.6% KH$_2$PO$_4$, 6.0% MgSO$_4$, 7.5% KCl and 2.5% FeCl$_3$, added with one of the four NaCl concentration levels (0, 50, 100 and 200 mM) for one month. Plants were harvested for physiological and biochemical analysis at 35 days after sowing for a total of 20 plants from each treatment.

**Plant growth parameters determination**

Plant growth (root dry weight, shoot dry weight, stems diameter (SD) and total leaf area (TLA)) was evaluated using twenty plants from each cultivar. All tissue parts (leaves, stems, and roots) were separated, and fresh weights of these tissue parts were measured. For the determination of dry weight, these tissue parts were dried at 65°C for 72 h. SD was measured every week on plants using a caliper. TLA was measured every week and calculated using the formula described by Kumar et al. (2002):

$$\text{TLA (cm}^2\text{)} = L \times I_a \times 0.80 \times N \times 0.662$$

Where $L$ = length of leaf; $I_a$ = width of leaf and $N$ = total number of leaves.

**Biochemical parameters determination**

Proline (PRO) content was extracted from fresh leaves according to the method of Bates et al. (1973). Leaves samples (0.5 g) were homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid to precipitate protein. Samples were centrifuged at 18,000 $g$ for 10 min and supernatant was used for estimation of PRO content. The reaction mixture consisted of 1 mL acid ninhydrin and 1 mL of glacial acetic acid, which was boiled at 100°C for 1 h. After tubes cooling in the ice, the products were extracted with 2 mL of toluene by vortex mixing and the upper (toluene) phase decanted into a glass basin. The absorbance was recorded at 520 nm and the PRO content was measured at 663 and 645 nm with a BECKMAN DU 68 spectrophotometer. A standard curve was established using chlorogenic acid. TP content was expressed as $\mu$g g$^{-1}$ FW.

**Measurements of the chlorophyll content**

Chlorophyll (CHL) content in leaves was estimated after extracting 20 mg of the ground material, following the procedure described by Arnon (1949). Chlorophyll of samples was extracted with 80% alkaline acetone (v/v). Full extraction of chlorophyll was achieved when the sample was discoloured. The absorption of the extracts was measured at 663 and 645 nm with a BECKMAN DU 68 spectrophotometer and CHL was calculated using the following formula:

$$\text{Total chlorophyll} = (20.2 \times D_{645} + 8.02 \times D_{663}) 	imes (50/1000) \times (100/5) \times 1/2$$

Where, D = absorbance and expressed as mg g$^{-1}$FW.

**Statistical analysis**

Results obtained from all the manipulations are expressed as mean ± standard deviation and analyzed using SPSS software. Statistical differences between treatment means were established using the Fisher LSD test at $p$ values $< 0.05$. Multifactorial ANOVA was used to estimate whether cultivar, salinity level, alone or in interaction, had a significant influence on the measured parameters.

**RESULTS**

**Growth parameters**

Peanut growth was estimated by measuring root dry weight (RDW), shoot dry weight (SDW), total leaf area (TLA) and stem diameter (SD) (Table 1). A significant two-way interaction between the factors, salinity level and cultivars, was observed for SDW and TLA (Table 1). The RDW and SDW of cv. Vanda decreased significantly ($p<0.05$) in salt-treated plants, when compared with control plants than those of cvs. Fleur 11, Mbiah and PC 79-79. The SDW inhibition effect of salt was notably noted at 100 mM NaCl in cvs. Mbiah and PC 79-79, while cv. Fleur 11 showed significantly ($p<0.05$) decrease in salt-treated plants only at 200 mM NaCl but had higher SDW accumulation than others (Table 1). TLA of all cultivars was negatively affected with increasing levels of salinity (Table 1). At the highest salt concentration (200 mM
NaCl), after four weeks of salt treatment, TLA was strongly reduced in cv. Vanda compared to control plants than those of cvs. Fleur 11, Mbiah and Pc 79-79. SD of all cultivars was not affected by salinity levels except for Pc 79-79 and Vanda at high salinity level (200 mM) after four weeks of salt treatment (Table 1). In general, plant growth was influenced by NaCl treatment except for SD and the magnitude of responses varied according to cultivars differing in salt-tolerance (Table 1).

**Biochemical characteristics**

Proline (PRO) content in leaf of control and NaCl stressed plants of all peanut cultivars were found at 35 DAS and results are presented in Figure 1a. The PRO content was significantly increased in the stressed plants compared to control plants of all cultivars at all salinity levels but differences in PRO accumulation have been noticed during plant growth between peanut cultivars. The highest increase was observed in cv. Fleur 11 while the lowest was found in cv. Vanda; cv. Mbiah maintained higher increase of PRO content than the cv. PC 79-79.

Glycine betaine (GB) content was determined in the absence (non-saline control) as well as in presence of NaCl in leaves of all the peanut cultivars after 4 weeks of salinity treatment (Figure 1b). GB content significantly (p<0.05) increased in presence of NaCl in all the cultivars compared to control plants. This increase was more pronounced in cv. Fleur 11 than others, while cv. Vanda showed the lowest increase.

**Changes in the chlorophyll content**

Leaf total chlorophyll content (CHL) was substantially increased in cvs. Fleur 11 and PC 79-79 with increasing

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Salinity level (mMNaCl)</th>
<th>Plant dry weight (g plant⁻¹)</th>
<th>Stem diameter (cm)</th>
<th>Total leaf area (cm² plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td></td>
</tr>
<tr>
<td>Fleur 11</td>
<td>0</td>
<td>0.74±0.04b</td>
<td>0.11±0.01b</td>
<td>0.27±0.01a</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.78±0.02a</td>
<td>0.14±0.02a</td>
<td>0.27±0.02a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.75±0.03b</td>
<td>0.14±0.02a</td>
<td>0.26±0.03b</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.50±0.02d</td>
<td>0.12±0.01ab</td>
<td>0.25±0.02a</td>
</tr>
<tr>
<td>Mbiah</td>
<td>0</td>
<td>0.65±0.02c</td>
<td>0.10±0.03b</td>
<td>0.27±0.02a</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.68±0.02b</td>
<td>0.12±0.03ab</td>
<td>0.27±0.02a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.58±0.02c</td>
<td>0.14±0.02a</td>
<td>0.26±0.03b</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.43±0.01g</td>
<td>0.07±0.03f</td>
<td>0.25±0.01b</td>
</tr>
<tr>
<td>PC 79-79</td>
<td>0</td>
<td>0.70±0.04c</td>
<td>0.15±0.05a</td>
<td>0.27±0.01a</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.67±0.05c</td>
<td>0.14±0.04a</td>
<td>0.27±0.03a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.43±0.03g</td>
<td>0.14±0.02a</td>
<td>0.26±0.04a</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.34±0.03h</td>
<td>0.12±0.01ab</td>
<td>0.24±0.02ab</td>
</tr>
<tr>
<td>Vanda</td>
<td>0</td>
<td>0.74±0.01b</td>
<td>0.15±0.03a</td>
<td>0.26±0.01a</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.63±0.03cd</td>
<td>0.06±0.01c</td>
<td>0.25±0.02ab</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.37±0.01h</td>
<td>0.05±0.01c</td>
<td>0.24±0.02ab</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.26±0.01i</td>
<td>0.03±0.00cd</td>
<td>0.23±0.01b</td>
</tr>
</tbody>
</table>

**Table 1.** Peanut cultivars growth parameters at different salinity levels. Data are mean ± standard error (n =5).

Within columns, means followed by the same letter are not significantly different (p< 0.05) by Fisher LSD test; *, **, *** Significant at p< 0.05, p< 0.01 and p< 0.001 respectively, ns not significant.
NaCl concentrations (Figure 2). Salt stress caused gradual reduction in CHL content of cv. Vanda but showed no change and decreased in cv. Mbiah at 50 and 100 mM NaCl, respectively, compared to control plants. Under stressed conditions, CHL content in cv. Vanda was lower than that in other cultivars.

**DISCUSSION**

**Plant growth**

Depletion in plant growth (RDW and SDW) under saline stress in cv. Vanda than cvs. Fleur 11, Mbiah and PC 79-79 after 4 weeks of salt treatment (Table 1) is attributed to decreased water uptake followed by limited hydrolysis of food reserves from storage tissue, as well as due to impaired translocation of food reserves from storage tissue to the developing embryo axis (Meneguzzo et al., 1999). Numerous studies have reported the reduction of RDW and SDW stimulated by salinity (Taffouo et al., 2009; Zahra et al., 2012; Navarro et al., 2014). The reduction in growth parameters is a consequence of several physiological responses including modification of ion balance, mineral nutrition, stomatal behaviour and photosynthetic efficiency also (Rajest et al., 1998; Dadkhah, 2011). This is consistent with the reports that NaCl reduces the ability of the plant to take up water, and this leads to slow growth and then, when excessive amounts of salt entering the transpiration stream will eventually injure cells in the transpiring leaves and this may further reduce growth (Munns et al., 2006). In the present study, the growth inhibition effect of salt in growth parameters studied was significantly noted at 50 mM NaCl in cv. Vanda than others, while the growth of cv. Fleur 11 was significantly (p < 0.05) affected only at 200 mM NaCl. These results demonstrate that cv. Vanda, in common with certain other bambara groundnut cultivars (black seed coat and light red seed coat), is highly sensitive to salt with severe effects at 50 mM NaCl (Levitt, 1980). Under salt stress cv. Fleur 11 was observed to have relatively higher tolerance on average.
of all growth parameters than others. Similar observations for plant growth were reported in *Ceriops roxburghiana* (Rajest et al., 1998) and *Vigna subterranea* cv. white seed coat (Taffouo et al., 2010a) described as salt-tolerant plant species. In the first phase of a biphasic model of growth response to salinity, the vegetative growth is reduced by a decrease in a soil water potential due to water stress effect and may be regulated by inhibitory signals from the roots (Munns et al., 1995).

**Biochemical characteristics**

The result of the present study shows that the proline (PRO) content was significantly (p < 0.05) increased in the stressed plants compared to control plants of all cultivars at all salinity levels but differences in PRO accumulation have been noticed during plant growth between peanut cultivars (Figure 1a). The highest increase, which was observed at 200 mM NaCl in cv. Fleur 11, is consistent with numerous studies which found accumulation of PRO in salt tolerant plants exposed to salt stress (Sivaramakrishnan et al., 1988; Meloni et al., 2004). A positive correlation between magnitude of PRO accumulation and salt tolerance has been suggested as an index to determine salt tolerance potentials between cultivars (Ramanjulu and Sudhakar, 2001; Giridara Kumar et al., 2003; Grigore et al., 2011). By contrast, it has been also reported that the salt sensitive cultivars accumulated significantly higher levels of PRO compared to tolerant ones (Lutts et al., 1999; Vaidyanathan et al., 2003). In this study, we report a significant difference in PRO accumulation between salt-tolerant cv. Fleur 11 and salt-sensitive cv. Vanda during plant growth. The PRO increases with increasing salinity are an adaptative change in metabolism pattern (Mudgal et al., 2010). Previously, it has been reported that Na\(^+\) and Cl\(^-\) are efficient osmolytes for osmotic adjustment and sequestered in the vacuole of a cell in salt-tolerant species, the osmotic balance of the cytoplasm is ensured by an active synthesis of the organic and soluble compounds (Le Rudulier, 2005; Grigore et al., 2011). This synthesis of the PRO is a mechanism of stress resistance (Greenway and Munns, 1980), because its accumulation contributes to the acquisition of resistance by maintaining the cell turgor in many species which is responsible for the osmotic adjustment in susceptible plants under stress (Meloni et al., 2004). The species growing in arid or semi-arid lands, may exhibit more tolerance to salt stress, because of the accumulation of osmo-protectants in their tissues (Munns, 2002).

In the present investigation, it has been noticed that the highest accumulation of glycine betaine (GB) was found in salt-tolerant cv. Fleur 11 than others, while the lowest was observed in salt-sensitive cv. Vanda (Figure 1b). GB is one of many nitrogenous osmolytes accumulated under osmotic stress conditions in salt-tolerant plants (Girija et al., 2002). Numerous studies reported that the accumulation of GB was found to be high in salt-tolerant cultivar (Rhodes et al., 1987; Giridara Kumar et al., 2003), while salt-sensitive cultivar exhibited a low magnitude of GB accumulation (Hitz and Hanson, 1980). GB preserves thylakoid and plasma membrane integrity of Zea mays after exposure to saline solutions (Rhodes et al., 1987). Thus, the accumulation of osmo-protectants in tissues of plants growing in arid or semi-arid lands, may

![Figure 2. Total chlorophyll content in leaves of peanut cultivars in response to NaCl concentration levels. Data are mean ± standard error (n =5). Means followed by the same letter are not significantly different (p <0.05) as determined by Fisher LSD test. Bars indicate standard error.](image-url)
exhibit more tolerance to salt stress (Munns, 2002). Salt stress can lead to stomatal closure, which reduces CO₂ availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn could increase the generation of Reactive Oxygen Species (ROS) and induce oxidative stress (Parvaiz and Satyawati, 2008). The osmo-protectants that accumulate most commonly are PRO and GB, although other molecules can accumulate to high concentrations in certain species (Girija et al., 2002).

NaCl salinity induced total phenolic content (TP) accumulation in leaves in all cultivars compared to control plants (Figure 1c). TP accumulation in leaves under salt stress could be a cellular adaptive mechanism for scavenging oxygen free radicals during stress conditions (Mohamed and Aly, 2008). Numerous studies have reported that TP production is stimulated by NaCl (Han et al., 2008; Zahra et al., 2012). Antioxidants prevent lipid oxidation and can act in different ways, including decreasing oxygen concentrations, scavenging initiating radicals, and binding metal ions to prevent initiating radical formation (Dorman et al., 2003).

In this study, we present the evidence that salt stress is able to produce excessive quantity of malondialdehyde (MDA) in the leaf of cv. Vanda plants than others cultivars (Figure 1d). Increase in the level of MDA, produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage (Azad et al., 2012). Free radical-induced peroxidation of lipid membranes is a reflection of stress-induced damage at the cellular level (Nagest and Devaraj, 2008). As a sequel, lipid peroxidation products such as MDA will accumulate and severe membrane damage will inevitably occur (Azad et al., 2012). Previously, it has been reported that there was an improvement in MDA content in leaves of salt-sensitive Vigna radiata and Plantago media, but decreased at 200 mM in salt-tolerant Plantago maritima under salinity (Sekmen et al., 2007; Saha et al., 2010). MDA level and cell membrane damage increased under salt stress condition in salt-sensitive cv. Vanda because of elevating of ROS production (Azad et al., 2012). MDA concentration decreased at 200 mM in salt-tolerant cv. Fleur 11. These results suggested that the cv. Fleur 11 showed a better protection mechanism against oxidative damage caused by salt stress by its higher induced activities of antioxidant enzymes than the salt-sensitive cv. Vanda (Sekmen et al., 2007).

Changes in the chlorophyll content

Salinity decreased the chlorophyll (CHL) content in salt-sensitive cv. Vanda (Figure 2) leaves, which is in accordance with Tafkou et al. (2010a; b), and Giannakoula et al. (2012). This effect of salt was attributed to a salt-induced weakening of protein-pigment-lipid complex (Strogonov et al., 1970) or increased chlorophyllase enzyme activity (Stivesev et al., 1973). By contrast, the CHL content was substantially increased in Fleur 11 and PC 79-79 with increasing NaCl concentrations. Similar observations have been reported by Robinson et al. (1983) and Morales et al. (1992) in salt-stressed spinach and barley, respectively. Moreover, the cultivars Fleur 11 and PC 79-79 screened for their salt tolerance in this study were grown under natural field conditions of Bamby (Senegalese) and were probably exposed to different environmental constraints. The interaction between these stresses could be taken as a part of the adaptive mechanisms of plants to survive under saline conditions and high temperatures (Giannakoula et al., 2012).

Conclusion

In general, the results of this study showed that salt stress caused a serious decrease in plant growth by means of reduced RDW, SDW and TLA due to ionic toxicity and decrease osmotic potential in all peanut cultivars but the magnitude of responses varied according to cultivars. Higher osmolyte accumulation, especially proline and glycine betaine was found in the salt tolerant cultivar (Fleur 11) whereas the lower in salt-sensitive one (Vanda). The salt stress was able to excessively generate MDA in the leaves of Vanda plants, that is, an indicator of oxidative damage. MDA levels increased under salt stress in the leaves of cv. Vanda but decreased in cvs Mbiah, PC 79-79 and Fleur 11 at 100 and 200 mM, respectively. These results suggest that Fleur 11 exhibits a better protection mechanism against oxidative damage caused by salt stress due to its higher induced activities of antioxidant enzymes and osmolyte accumulation than Mbiah, PC 79-79 and Vanda. Fleur 11 can tolerate moderate saline conditions owing to better antioxidant system. It seems that the evaluation of osmolyte accumulation and antioxidant system is useful for assessment of salinity tolerance of peanut cultivars.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

Liliane Tekam Meguekam has received a half-time doctoral fellowship from AUF-Eugen Ionescu. This work was financially supported by the AUF-Eugen Ionescu 2011 and 2012 scholarship research programme.

REFERENCES


Didactic and pedagogic publishing House Edition, Bucharest.


Full Length Research Paper

Antidiabetic activity and acute toxicity evaluation of aqueous leaf extract of *Vernonia amygdalina*

M. A. Momoh¹, M. O. Adedokun²*, A. T. Mora³ and A. A. Agboke²

¹Drug Delivery Research Unit, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria.
²Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
³Department of Clinical Pharmacy and Pharmacy Management, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna, Nigeria.

Received 19 June, 2014; Accepted 28 November, 2014

The present study was aimed to explore the therapeutic dose for antidiabetic activity and toxicological evaluation of *Vernonia amygdalina* (Va) aqueous extract in alloxan-induced diabetic rats. Aqueous extract of leaves of Va was administered to alloxan induced diabetic rats in a dose of 150 and 300 mg/kg (orally) daily for 14 days. After this period, blood glucose level, haematological parameters and liver enzymes activities were evaluated. Also evaluated were food and water intake, urine output and body weight of the animals. The toxic effect of the aqueous leaves extract was evaluated by determining the LD₅₀. Oral administration of the extract at graded doses of 150 and 300 mg/kg body weight showed significant decrease in the blood glucose level in diabetic rats (P<0.05). The defects in haematological and enzyme activities in the diabetic animals were restored. We concluded that at doses of 150 and 300 mg/kg, Va extract exhibited anti-hyperglycemic effect and showed statistically significant differences (p<0.05) in all the parameters evaluated. There was a significant improvement (P<0.05) in the weight of the diabetic rats, food intake and a decrease in the urine output. This study illustrates the potential usefulness of this extract and its safety on a vital organ of the body.

Key words: Alloxan, *Vernonia amygdalina*, diabetics, aqueous extract, rats, safety.

INTRODUCTION

Diabetes mellitus is the clinical condition of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Obimba et al., 2014; Ozougwu et al., 2013). The chronic hyperglycemia caused by diabetes is associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (International Diabetes Federation (IDF), 2009). There are two types of diabetes: Type 1 diabetes, previously called insulin-dependent diabetes mellitus or juvenile-onset diabetes which accounts for up to 10% of all diagnosed diabetes which is caused by lack of insulin secretion by β-cells of the pancreas. Type 2 diabetes was previously called non-insulin-dependent diabetes mellitus or adult-onset diabetes which may account for between 90 to 95% of all diagnosed cases of diabetes (Obimba et
al., 2014; Ozougwu et al., 2013). Type 2 diabetes mellitus has become a significant health problem in both developed and developing countries. IDF has estimated that 285 million people around the world have diabetes. This total is expected to rise to 438 million within 20 years (World Health Organization, (WHO), 2003). The non-pharmacological means (diet and exercise) and/or the pharmacological means (insulin and oral hypoglycaemics) have been used in the management of diabetes mellitus. The obvious limitations of oral administration of antidiabetic agents, especially with insulin, have necessitated a search for alternatives among the arsenal of herbs available to man (Edwin et al., 2006). A large number of herbs, spices and other plant materials have been described for the management of diabetes throughout the world (Ojiako and Nwanjo, 2006). It was in this light that the World Health Assembly adopted among its resolutions the support of national traditional medicine program, drawing attention to herbal medicines as being of great importance to the health of individuals and communities (Emeje et al., 2011).

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived from plants. Vernonia amygdalina (family, Compositae) is a valuable shrub that is wide spread in East and West African countries (Izievbigie, 2003). In Nigeria, it is commonly known as “bitter leaf” because the leaves and stem have a bitter taste when chewed. Its leaves were used as a popular vegetable for soups particularly among the ethnic groups in Nigeria. The organic fraction extracts of the plant was shown to possess cytotoxic effects on human carcinoma cells of the nasopharynx (Okolie et al., 2008). It is effective against gastrointestinal disorders (Akah and Ekekwe, 1995) including amoebic dysentery (Moundipa et al., 2005) possibly due to its antimicrobial and antiparasitic activities (Gray et al., 2000; Muraina et al., 2010). Its antithrombotic and anticoagulant properties have also been evaluated (Akah and Ekekwe, 1995). Oral administration of the aqueous leaf extract of the plant was found to relieve pain and to lower body temperature (Gray et al., 2000). V. amygdalina is also used traditionally as an antidiabetic remedy in Nigeria by the traditional herbal practitioners (Muraina et al., 2010; Park, 2007; Atawodi, 2005). Several workers have provided some scientific proofs in support of this practice (Abdulazeez et al., 2013; Iwuji et al., 2010) but, available scientific reports on the actual dose needed for management of diabetic conditions and the possible mechanism of action of this plant were not detailed enough. Thus, we conducted this study to further investigate the effective dose for antihyperglycemic effect and to a less extent its safety on a vital organ of the body.

**MATERIALS AND METHODS**

**Preparation of crude extract**

Pesticide-free fresh leaves of V. amygdalina were collected from Nsukka, Enugu State, Nigeria and authenticated at Bioresources Development and Conservation Program (BDCP), Nsukka, Nigeria where voucher samples were kept for reference. Healthy fresh leaves were sorted, washed to remove debris and dust particles without squeezing and then air-dried for seven days (Momoh et al., 2013). The dried leaves were milled into a coarse powder from which 25 g was soaked with 500 mL of distilled water in a beaker and the mixture shaken on the laboratory bench for 24 h before filtering (Whatman No. 1 filter paper). The filtrate was evaporated using a rotary evaporator to obtain a solid residue (5.0 g) called the aqueous extract which correspond to a percentage yield of 20%. This procedure was carried out in quadruplicate to obtain a total of 20.0 g of aqueous extract. Appropriate weights of the residue were reconstituted separately in distilled water to give the required doses of 100, 150 and 300 mg/kg body weight used in the present study.

**Phytochemical tests on the extract**

Some phytochemical tests were carried out on the V. amygdalina extract to determine the presence of saponins, flavonoids, alkaloids, proteins, carbohydrates, glycosides and tannins using standard methods (Sofowora, 2006).

**Preliminary evaluation of hypoglycemic activity of extract in normal healthy albino rats**

Twenty normal Albino rats, fasted overnight, were divided into four groups of five rats each, and used in the experiment. Group A served as control, and received the vehicle (distilled water only) while animals of groups B, C and D received variable doses of 100, 150 and 300 mg/kg, respectively, of extract suspended in distilled water. The blood samples were collected from the lateral vein of the tail of the animals at 2, 4, 6, 8 and 12 h after administering the extract and the sugar levels were measured. These were compared to the initial blood glucose level.

**Experimental design**

Albino rats (180 to 220 g) of either sex were used for the study. The animals were procured from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, and were allowed to acclimatize to the new environment for a period of two weeks prior to the study. Rats housed in cages were kept in a room with controlled temperature (20 to 22°C) and a 12 h day-night cycle. Fresh solution of alloxan monohydrate (Sigma, USA) was prepared just prior to injection. A stock solution of it was made by dissolving all in normal saline (0.9% w/v NaCl) at a concentration of 100 mg/mL (Dhaasaranth and Theriappan, 2011). After being starved overnight, a volume equivalent to 1 mL of the stock solution was administered intra-peritoneally to the animals after which the blood glucose levels were measured for days using a glucometer (ACCU-CHEK, Roche, USA). Food consumption, water intake and urine volume were similarly evaluated daily. The rats were considered diabetic when the blood glucose level was raised above 200 mg/100 mL of blood (Cetto et al., 2000) after 3 days post-alloxan administration. All of the animal experiments adhered to the Ethical Guidelines of Animal Care and Use Committee (Research Ethics Committee) of Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria.

**Oral administration of graded dose of extract to diabetic rats**

Twenty (20) Albino rats were used for the study. They were divided into four groups of five rats per group. Based on an initial normoglycemic study, 150 and 300 mg doses were selected for the...
antidiabetic evaluation. The first group marked A, received distilled water (negative control, 1.5 mL orally). The groups marked B and C received the extract at 150 and 300 mg dispersed in distilled water (1.5 mL orally), respectively, while that marked D received glibenclamide dispersed in distilled water (2.0 mg/Kg orally). The extract was administered once daily by gastric intubation for 14 consecutive days.

Acute toxicity evaluation

The acute toxicity studies were carried out based on Lorke’s method (1983) (with slight modifications) on adult male Wastar rats of an average weight of 220 g. The animals were housed in air-conditioned quarters under a photoperiod schedule of 12 h light/12 h dark. They were fed on standard animal pellets and had free access to water ad libitum. The rats were randomized into two groups (i and ii) of five rats each and were orally administered graded doses of the extract 3,000 and 6,000 mg/kg body weight, respectively. The doses were 20 times the most effective dose of the aqueous extract of *V. amygdalina* used in the antidiabetic evaluation. They were observed for signs of toxicity that included paw-licking, stretching, response to stimuli and mortality for the first 4 h and thereafter daily for seven days. Food consumption, water intake and urine output were also examined for 24 h. The lethal dose was calculated as the geometric mean of doses that caused 0 and 100% mortality, respectively.

Effect on liver enzymes and haematological parameters

Haematological parameters

Haematological analysis was performed using an automatic haematological analyzer (Abacus Junior, Germany). Haemoglobin (Hb) count, total white blood corpuscles (WBC) and packed cell volume (PCV) were specifically determined.

Liver enzymes activity

A 3 ml volume of blood collected in a plain bottle or serum extractor was used for the study. The blood was allowed to stand in an undisturbed bench for 1 h away from sunlight; this was followed by spinning for 5 min. Serum was separated from the clotted red cells, and the resulting supernatant used for the assessment of liver integrity. Using a 3.2 mL automated pipette serum was dropped on the sample spot of each LFT parameter strips (ALP Aspartate and Alanine aminotransferases strip) and analysed using a Reflotron-Plus machine (Model: SN747461).

Statistical analysis

Statistical analysis was performed using SPSS statistical package. Mean and standard errors for all data were calculated. For batch comparisons, the Student’s t-test was used to determine statistically significant differences at P≤0.05.

RESULTS

The results from the study showed that the aqueous leaf extract of *V*.* amygdalina* extract was positive for alkaloids, saponins, flavonoids, phenolics, tannins, cardiac glycosides and steroids (Table 1).

Effect of extract on fasting blood glucose level of normal healthy rats

Results in Table 2 reveal the hypoglycemic effect of graded doses of aqueous extract of *V. amygdalina* leaves on blood glucose level (BGL) of normal rats. Rats treated with 300 mg/kg of extract showed a maximum fall of 2.0% in BGL at 8 h of oral administration, whereas reduction of less than 2.0% were observed with the doses of 100 and 150 mg/kg, respectively, at 12 h of administration. Statistically, the extract produced no significant decrease on the BGL of normoglycemic (non-diabetic) rats (P>0.05).

Effect on the pre-and post polytriads symptoms and on body weight

As shown in Table 3, there were significant differences in

<table>
<thead>
<tr>
<th>Test</th>
<th>Present/absence</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>Present</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>-</td>
<td>Absent</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>++</td>
<td>Present</td>
</tr>
<tr>
<td>Anthracene glycosides</td>
<td>-</td>
<td>Absent</td>
</tr>
<tr>
<td>Steroidal glycosides</td>
<td>++</td>
<td>Present</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>Present</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
<td>Present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>Present</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>Present</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>Present</td>
</tr>
</tbody>
</table>

+ indicates presence of photochemical secondary metabolite; - indicates absence of phytochemical secondary metabolite.
Table 2. Effect of graded doses of aqueous extract of *Vernonia amygdalina* leaves on normoglycemic rats (mean ± S.D, n=3).

<table>
<thead>
<tr>
<th>Batch/treatment</th>
<th>Dose mg</th>
<th>Blood glucose level (%) at times (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>A (negative control)</td>
<td>DW</td>
<td>100</td>
</tr>
<tr>
<td>B (extract)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>C (extract)</td>
<td>150</td>
<td>100</td>
</tr>
<tr>
<td>D (extract)</td>
<td>300</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3. Effect on polytriad symptoms and on body weight before and after treatment with the extract in normo and hyperglycemic rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before treatment</th>
<th>After treatment with extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normoglycemic</td>
<td>Diabetic</td>
</tr>
<tr>
<td>Urine output (ml)</td>
<td>6.20±1.2</td>
<td>97.1±1.4a*</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>180±0.4**</td>
<td>114±0.2a*</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>23.0±0.6</td>
<td>83.0±2.3a*</td>
</tr>
<tr>
<td>Water intake (ml)</td>
<td>21.0±1.3*</td>
<td>123±1.2a*</td>
</tr>
</tbody>
</table>

a* Indicates significant difference at p < 0.05 among the diabetic treated group, and ** values were not significant (P>0.05) when compared to non-diabetic before and after treatment. All the treated diabetic rats showed significant improvement (P>0.05) compared to the normoglycemic rats before and after. Values are mean ± SD (n = 3).

all the parameters evaluated (water and food intake, urine volume output, and weight gain) between the groups of diabetic and non-diabetic animals. Studies have shown that food intake, urine output and decrease in body weight are associated to diabetes conditions due to impaired metabolic pathway that greatly affected the physiological wellbeing of the body. In this research, aqueous extract of *Vernonia amygdalina* administered to the diabetes groups successfully restored the anomalies comparably to the control normoglycemic rats. The food consumption of the diabetic rats increased approximately 15%, although these animals showed less body weight (weight = 114.0 g) compared to the group of non-diabetic animals (body weight = 180.2 g). Treatment of the diabetic animals with *Vernonia amygdalina* extract led to a significant change (P>0.05) in their consumption of food and water as well as the urine output and gain in body weight, comparable to the untreated non-diabetic rats (before administration of the extract). After the administration of the extract, there was a significant increase (P<0.05) in the body weight of the diabetic group comparable to the body weight of the control group. However, there were no significant change (P>0.05) in the urine output, food intake and gain in the body weight of the normoglycemic group after treatment with the aqueous extract (Table 3). The results clearly indicate that the diabetic conditions which cause an increase of food and water consumption and urine output, which ultimately lead to weight loss were generally reversed after the treatment. On physical examination, the changes in non-diabetic and diabetic rats were apparently distinctive because, apart from the thinness of diabetic rats, the rats also maintained a quiet look and showed a slow response to external stimuli, that is, touch. All these observations were restored in all the treated groups regardless of the concentration of the extract administered. However, the recovery rate was dose-dependent.

**In vivo experiment**

As shown in Figure 1, *Vernonia amygdalina* extract orally administered in diabetic rats allowed a significant decrease of glycemia compared to rats treated with water alone. The rats that received DW continued to have elevated blood glucose levels within the first 4 days, which may be due to force feeding as this can increase the glucose level. The decrease in the blood glucose level that was observed was due to the fasting conditions which caused a decrease in the blood glucose level. The extract (150 and 300 mg) dose-dependently lowered the blood glucose levels of the rats. Maximum blood glucose lowering (100 to 51%) was encountered in group C that was treated with 300 mg of the extract on the 6th and 12th day, and was comparable to the blood glucose reduction (100 to 54%) encountered in the group received a standard drug (glibenclamide) at the 4th days and later continued to show a slight increase throughout the period of this investigation (Figure 1). Although, it was observed that the standard drug showed relatively more antihyperglycemic effect within 4 days, the extract was able to maintain a persistent steady decrease in BGL in the
glycemic state, which is one of the desired effects in the clinical management of diabetic conditions.

Effect of the extract on haematology and liver enzymes

Previous studies have shown that the investigation of the acute toxicity is the first step in the toxicological study of an unknown substance. Although, the plant in question has been in use as a source of food in Nigeria and many other African countries, it is very important that the safety of this plant should be evaluated before proceeding to the formulation proper and also to provide the scientific justification for the medicinal utilization of this plant. The result of the haematological evaluation on the extract based on the evaluated parameters (PCV, WBC and Hb) showed no significant changes ($P>0.05$) in the hematological and liver enzymes activities as shown in Table 4. Diabetic conditions resulted in the decrease of the haematological parameters such PCV, Hb and WBC due to glycosylation that occurred as a result of excess sugar in the blood as seen in the diabetic control (DC) group.
DISCUSSION

Throughout the world, diabetes is the fastest growing metabolic disorder and is considered as a heterogeneous group of diseases characterized by major causes affecting cardiovascular, renal, neurological and ophthalmic systems (Chakkarwar and Manjrekar, 2005). Currently available synthetic oral antihyperglycaemic agents may be associated with an increased risk of unwanted effects on prolonged use (Edwin et al., 2006). So, there is need to investigate newer herbal medicines which have less side effects, easy availability and are economical (Shah et al., 2006). The age long use of herbal medicines in the management of diabetic conditions is a common practice in many countries across the globe including Nigeria. Therefore, the need to substantiate the folkloric claim of *V. amygdalina* as an antidiabetic agent using rat models is imperative. The results show that there was statistically significant P reduction (P<0.05) not only in the glucose level but also in the associated polytriads symptoms. Other related complications including weight loss were also improved significantly (P<0.05) in the treatment groups over time as compared to the negative control in the present study. Alloxan became the first diabetogenic chemical agent when scientists accidentally produced islet-cell necrosis in rabbits while researching the nephrotoxicity of uric acid derivatives. Alloxan is a specific toxin that inactivates the pancreatic β cells, provoking a state of primary deficiency of insulin without affecting other islet types (Akah and Okafor, 1992). Hence, alloxan was selected to induce diabetes in the present study. Based on a preliminary study, batches B and C were used for the *in vivo* study. As shown in Figure 1, the decrease of glycemia started within the first few hours after oral administration of the extract. This lag time could be due to the time required for *V. amygdalina* extract to reach the site of the gastrointestinal tract where the active constituent of the extract could be absorbed. The glycemic profiles indicate that both doses of extract and the conventional tablet displayed similar biological activity. The hypoglycemic action of extract (150 and 300 mg) and the positive control demonstrated rapid onset time of 2 days reaching maximal activity within 12 days. No biological effect was seen in the group treated with distilled water (DW) alone, the slight reduction in the blood glucose level was due to the effect of long term glycemia. It is well known that prolonged glycemia can cause a slight decrease in blood glucose level (Ojiako and Nwanjo, 2006; Abdel-Bary et al., 1997). As illustrated in Figure 1, both 150 and 300 mg doses administered significantly decreased blood glucose levels (P< 0.05) compared to the non-treated group.

The real mechanism by which the extract performed its action was not clear. The results obtained from the present study clearly confirmed that the tested extract possesses marked hypoglycemic activity on the alloxan-induced diabetic and non-diabetic rats. From the present experimental results, it could be suggested that, the extract exhibited dose dependent glucose lowering effects. The result of glucose lowering potentials of the extract is consistent with earlier reports on the hypoglycaemic action of the extracts of *V. amygdalina* in rats (Samy and Gopalakrishnakone, 2007). Nimemibo-Uadia (2003) attributed this action to tannins present in the extract of *V. amygdalina*, whereas other researchers presupposed a mechanism unrelated to insulin secretion from pancreatic β cell (Ebong et al., 2006). Be that as it may, it is probable that the two mechanisms may exist; one related to insulin production and the other targets peripheral carbohydrate metabolism. The former endows it with the ability to exert hypoglycaemia in diabetic rats, whereas the later achieves hypoglycaemia in non-diabetic rats. Alloxan is known to mediate pancreatic β-cells destruction via reactive oxygen species (ROS) generation (Szkudelsiki, 2001), depriving the animal of insulin, hence causing diabetes. *V. amygdalina* water extract having the ability to abate this alloxan-induced diabetes must necessarily have a corrective impact on the hitherto destroyed β-cells of the pancreas. It is possible to suppose that the antioxidant effect reported by Igile et al. (1994) to include luteolin, 7-O-beta glucoronoside and luelin, 7-O-beta glucoside may have attempted to reverse the cytotoxic effect of alloxan or at least to mop up the free radicals generated by alloxan responsible for beta cell destruction. By so doing, the β-cells could have started a gradual regeneration, hence insulin production commences to start an effectual control of hyperglycemia. On the other hand, the phytochemicals-endowed *V. amygdalina* may possess some alpha-glucosidase inhibitors as secondary plant metabolites. Such metabolites may competitively inhibit intestinal brush border enzymes-glucosidase, as well as pancreatic beta-amylase with the ultimate reduction in digestion and subsequent absorption of carbohydrates from the gut (Igile et al., 1994). Other researchers have proposed a parallel mechanism for tannins in their explanation (Igile et al., 1994). These were further strengthened by a researcher (Winleman, 1998), who indicated a strong positive correlation between the presence of flavonoids glycosides and phytosterols in plants and hypoglycaemic and antihyperglycemic activities, respectively. It is probable that the *V. amygdalina* extract may be endowed with both. In a related research by Ali et al. (1993), the author observed similar result in a different extract and the author resolved that the anti-diabetes effect was due to the alkaloid present in the plant.

Toxicological evaluation is an extremely important part of herbal formulation and development. High dose of extract is usually used for the study (Momoh et al., 2013). At the dose used for this evaluation, no toxic effect was observed on treatment with excess dose 20 to 30 times higher than the effective dose used in blood glucose level reduction. The physiological behavior of the rats remained
normal, there was no record of death and there was no sign of reduced activity after the administration of the 6,000 mg/kg, the response to stimuli (touch) were very good and sharp. The water and food intake were those not change compared to the negative control group. The oral LD₅₀ of the extract was estimated to be greater than 6,000 mg/kg body weight. Based on the result of this evaluation, we observed that this extract may be safe for human consumption. Pharmaceutical formulations for human consumption need to undergo safety evaluation in animal. This is a critical part of preclinical studies and forms an integral initial dossier during drug development. It is on these, that, clinical protocols, relevant instruction and contraindication and dosage are designed.

Hematological parameters are relevant to risk evaluation as the changes in haematological system have a higher predictive value for human toxicity, when data is translated from animal studies. The results in Table 4 indicated that there were significant changes (P>0.05) in the haematological parameters of the treated groups compared to the diabetic and non-diabetic control and groups. Levels of white blood cells (WBC), packed cell volume (PCV) and haemoglobin (Hb) studies increased following repeated administration of different concentrations of the extract. The result indicated that the extract was able to increase the secretion of the insulin which in turn halt gluconeogenesis and glycogenolysis which are serious factors in the loss of body weight in diabetic groups. The reversal of these pathways led to reduced glycosylation of the haemoglobin, hence the observed increase in the haematological parameters in the treated groups. Table 4 shows the results of the administration of graded doses (100, 150 and 300 mg) of extract of *V. amygdalina* on some liver function enzymes. The activities of both aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) increased in diabetic untreated group due to the liver damage which is associated with alloxan induced diabetes. The various concentrations of the extract (100 and 150 mg) significantly reduced the liver marker enzymes (AST, ALT and ALP) which was significant when compared with the control (P > 0.05). The decrease observed here is enough to encourage its use.

Contrary, there was a slight increase in AST in the group that received 300 mg of the extract, although this can be overlooked because, alanine aminotransferase (ALT) is a more reliable marker of liver integrity than aspartate aminotransferase (Bassey et al., 1987). The observed slight increase in the activity of aspartate aminotransferase alone may be of extrahepatic origin due to the effect of the hyperglycaemia. It has been observed that the more specific cytosolic ALT, found in high concentration in the liver and AST, which is localized in the cytosol and mitochondria are released into the circulation and may not be completely due to the dose of the extract administered (Reitman and Frankel, 1997).

**Conclusion**

Our results confirm the traditional use of leaf extracts of *V. amygdalina* in treatment of diabetes mellitus. The dose of extract of *V. amygdalina* used in this study, was very effective and safe in treatment of hyperglycemic condition, and equally restored the imbalance in liver enzymes markers and hematological parameters associated with diabetes condition. Consequently, this plant extract could be used alone or in combination with other agents in the management of diabetes. More so, it could also be recommended as a food supplement.

**Conflict of Interests**

The author(s) have not declared any conflict of interests.

**REFERENCES**


African Journal of Biotechnology

Related Journals Published by Academic Journals

- Biotechnology and Molecular Biology Reviews
- African Journal of Microbiology Research
- African Journal of Biochemistry Research
- African Journal of Environmental Science and Technology
- African Journal of Food Science
- African Journal of Plant Science
- Journal of Bioinformatics and Sequence Analysis
- International Journal of Biodiversity and Conservation