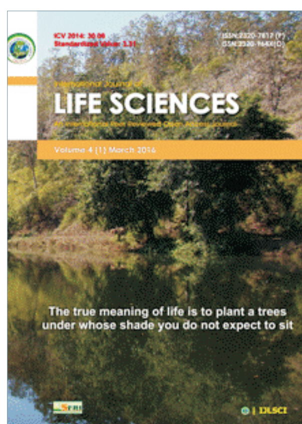
ICV 2014: 30.06
Standardized Value: 3.31

ISSN: 2320-7817 | e-ISSN: 2320-964X
Volume 4, Issue 1, March, 2016

INTERNATIONAL JOURNAL OF LIFE SCIENCES

AN INTERNATIONAL PEER REVIEWED OPEN ACCESS JOURNAL



Editor in Chief
Dr. Santosh S. Pawar

Managing Editor
Dr. Arvind Chavhan

STATUTORY WARNING

Articles, data, figures, scientific contents and its interpretation and authenticity reported by author(s) and published in IJLSCI are the exclusive views of author(s). The editorial board, IJLSCI is not responsible for any controversies arising out of them.
In case of any plagiarism found, author(s) will have to face its consequences.

All enquiries and manuscript should be directed to

The Managing editor IJLSCI
46, Guruvandan, Jawahar Nagar,
VMV Road, Amravati -444604,
(MS) India

Contact: +91-942 077 5527
+91-7249 600 511
Email: editor@ijlsci.in
editorijlsci@gmail.com
web:www.ijlsci.in

All the materials in this journal is copyright and may not be reproduced except with the return permission of the publisher
© Copyright International Journal of Life Sciences

About IJLSCI

International Journal of Life Sciences (IJLSCI) is a peer-reviewed, open access journal; it publishes original articles in all areas of Biology and Life Sciences. Authors are encouraged to submit complete unpublished and original works, which are not under review in any other journals. The scopes of the journal include, but limited to the following topic areas: Biology, Anatomy, Botany, Cytology, Genetics, Microbiology, Physiology, Zoology, Ecology, Environmental Science, Hydrobiology, Neurobiology, Developmental Biology, Immunology, Molecular Biology, Biochemistry, Biophysics, and Biotechnology, Genomics and Proteomics. It is an open access journal, which publishes original research articles, reviews and short communication in all areas of life sciences and at the interface of related disciplines.

TYPES OF RESEARCH PAPER

Original Articles:
These should describe new and carefully confirmed findings, and experimental methods should be given in sufficient detail for others to verify the work. The length of an original article is the minimum required to describe and interpret the work clearly.

Short Communications:
Short communication also contain abstract. The list of references should not exceed 15. The presentation of Short Communications should contain headings such as Introduction, Materials and Methods, Results and Discussion, etc.

Reviews Articles:
Submissions of review articles and perspectives covering topics of current interest are welcome and encouraged. Publication Frequency: Quarterly (published in March, June, September & December).

The journal is published in print as well as online version which is free access and downloads.

This Journal is circulated / Distributed Through Subscription only

Submission of Paper: Submission of paper is encouraged by e-mail to editorijlsci@gmail.com
Subscription Plan: Rs. 2500 /- or 150 US$ (Individual Annual); Rs. 3500/- or 250 US$ (Institutional)
Life Membership: Rs. 10000/- or 550 US$ (Individual) Rs. 15000/- or 850 US$ (Institutional)
Fellow Membership: Rs. 15000/- or 850 US$ (Individual) Rs. 25000/- or 1700 US$ (Institutional)

Publisher and Owner: **Dr. Arvind Chavhan,** Published from IJLSCI, 46, Guruvandan, Jawahar Nagar, VMV Road Amravati -444604, India

Printed at IJLSCI, 46, Guruvandan, Jawahar Nagar, VMV Road Amravati -444604, India

Managing Editor: **Dr.Arvind Chavhan**,Assistant Professor & Head, Department of Zoology, D.B. College, Bhokar, Nanded, India

Publisher, Editorial Board and Editor in Chief take no responsibilities for the inaccurate, misleading data, opinion and statement appeared in the articles published in this journal.
All responsibilities of the contents rest upon the authors (especially upon the corresponding author).

Indexing:

Index Copernicus, OCLCWorldCat, Directory of Science INNOSPACE (SJIF), ROAD, OAJI, AcademicKeys, CiteFactor, J-index.net, Scholar Steer, ResearchBible, Google Scholar, DAIJ , DRIJ, DIIF, SIS, ISI, ASI.

No Part of the this Journal can be reproduced without written permission of the publisher © Copyright 2013-15 By Dr. Arvind Chavhan, Managing Editor, International Journal of Life Sciences, Amravati- 444604. India

All Articles are freely accessible on the website of the International Journal of Life Sciences http://www.ijlsci.in As well as some of the Indexing agencies website via., oaji.net, research bible, Cite factors, ISI, etc.

IJLSCI

ISSN:2320-7817(p) | 2320-964X(o)
Volume 4, Issue 1, March 2016

INTERNATIONAL JOURNAL OF LIFE SCIENCES

Contents

ORIGINAL ARTICLES

1 Distribution, physiologic races and reaction of wheat cultivars to virulent races of leaf rust (Puccinia triticina Eriks and Henn.) in south eastern zone of Tigray, Ethiopia
Tesfay Gebrekirstos Gebremariam | Getaneh Woldeab | Thangavel Selvaraj

22 Nematicidal potential of three plant materials against root-knot nematode (Meloidogyne incognita) infestation on tomato (Solanum lycopersicum)
Ekpenyong EP | Ononuju CC | Orikara CC | Ikwunagu EA

29 Assessment of Phytochemicals and Antioxidant activities of Leucas indica aerial parts- A comparative study
Sowjanya M | Kiran Kumar M | Sandeep BV

44 An evaluation of comparative biosorption study of Cadmium (II) and Chromium (VI) using Orange rind (Citrus sinensis), (L.) Osbeck, under optimized conditions
Poojari Anukthi C | Maind Sandip D | Bhalerao Satish A

57 Effect of temperature on the antimicrobial activity of lime juice-honey syrup on certain bacterial isolate
Unegbu Chika C | Njoku SC | Ajah Obinna | Nnaoma IE

63 Effects of sunlight exposure vs. commercial vitamin D supplementation on the bone health of vitamin D deficient rats
Mahmoud Mustafa Ali Abulmeaty MD

71 Observation on the histochemistry of the developing ova in *Haemonchus contortus* (Nematoda)
Jatinderpal Singh

78 Histological structure of pancreas in normal control, diabetic control and extract treated Albino rats
Kinkar Shobha Bhanudas | Patil Kishor Gopal

83 Estimation of Gallic acid, Rutin and Quercetin in Portulaca quadrifida L. – A potential wild edible plant by HPTLC method
Marathe Vishal R | Umate Satish K

89 GIS- based morphometric analysis of major watersheds ofTehran- Karaj, Central of Iran
Sajjad Fazel Tavassol | Gopalakrishna GS

97 3, 5-Dichloroanthranilic acid (DCA) - an elicitor induces systemic resistance against downy mildew in pearl millet
Lavanya SN and Amruthesh KN

107 Diversity of benthic macro-invertebrates in four tributaries of River Narmada in the central zone, India
Golwalkar Rachna Deo | Sleeqa Banoo | Mustaq Tehmeena | Diwan Suniti | Kumar Ankit | Vyas Vipin

116 Ground water quality assessment and its impact with special reference to Chhindwara District of Madhya Pradesh, India
Neelima Bagde

121 Studies on effects of dietary aflatoxin on growth performance and survival rate of fish *Clarias batrachus*
Fatmi Amjad | Ruby Durreshahwar

125 Zooplankton Diversity of a freshwater Pond in a Cachar district of Assam, India
Kar S | Kar D

129 The ecological impact of invasive Trematode Parasites of Common toad (*Bufo melanostictus*) found in bank of Saryu River along Chapra town and effect of flanking population
Seema Kumari | Vishwranjan | Prashant Kumar

133 Changing biodiversity scenario in Lonar meteoritic crater, (MS), India, as revealed by the studies on insects (Order- Lepidoptera, Orthoptera and Odonata)
Pedge Sudarshan S and Ahirrao Sunil D

137 Studies of water borne E.coli from the Congo red medium differentiation between pathogenic & non pathogenic in the broiler farms in Madhya Pradesh
Mishra Priti | Ther Sandip V | Shukla Satish

RESEARCH REPORT

140 Cercarial fauna of Malabar, Kerala- I
Venugopalan Nambiar M

SHORT COMMUNICATION

145 Biodiversity of Avifauna in Mumbri Creek of South Konkan, Deogad Taluka, Maharashtra
Dekate HM and Baviskar RN

148 Penicillium expansum toxins correlate with post-harvest fruit rot of apple (Pyrus malus L.)
Baviskar RN and Dekate HM

REVIEW ARTICLES

151 Stem Cells: Spatio-temporal Diversity and Therapeutic Applications
Chaudhary Pankaj Kumar | Mishra Kamlesh Kumar Chaudhary Jitendra Kumar

159 Author Index

160 IJLSCIEVENT

IJLSCI

ISSN:2320-7817(p) | 2320-964X(o)

INTERNATIONAL JOURNAL OF LIFE SCIENCES

Editorial Board

Editor in Chief

Dr. Santosh Pawar
Department of Biology,
Institute of Forensic Sciences,
Nagpur, India

Managing Editor

Dr. Arvind Chavhan
Department of Zoology,
D.B. College, Bhokar, Nanded,
India

Associate Editor

Dr. Bela Volgyi
Department of Experimental Zoology
and Neurobiology, Pécs, Ifjúságu,
Hungary

Dr. Fazal Shirazi,
Anderson Cancer Center,
1515 Holcombe Blvd, Houston, Texas.

Dr. Gunjan Mukherjee, Ph.D.,
Department of Biotechnology and
Bioresources, (TERI)
Indian Habitat Centre (IHC),
New Delhi, India

Dr. Suresh B. Zade
Professor in Zoology,
RTM University, Nagpur, MS, India

Dr. Satish A. Bhalerao
Professor in Botany,
Department of Botany,
Wilson College, Mumbai, India

Dr. Mulchand M. Rathod
Professor in Botany,
S.S.M.M. Arts science and Comm
College, Pachora, Jalgoan, India

Dr. M.N. Bhajbhuje
Department of Botany,
Jawaharlal Nehru College,
Wadi, Nagpur, India

Dr. B.N. Pandey
P.G. Department of Zoology,
Purnea College, Purnia, Bihar, India

Dr. Kamble SY,
Dy. Director, Botanical Survey of India,
Pune.

Dr. Mali Rajendra Prabhakar
Department of Zoology,
Yeshwant Mahavidyalaya,
Nanded (MS) India

Associate Editor

Dr K . Madhava Chetty,
Plant Taxonomist, Department of
Botany, SVUCS, S.V. University,
Tiupai51 7 502, AP, Indi.

Dr. Gyan Singh Shekhawat
Department of Botany,
Jai Narain Vyas Unive rsity,
Jodhpur, India

Dr.Gupr eet Singh Dhillon
Institut national de la Recherche
Scientifique (INRS), Cente for Water,
Earth and Environment;
Univ ersity of Québec, CANADA

Dr. Bankefa Emmanuel Olufemi
Department of Microbiology,
Federal Univ esity of Technology,
Akure, Ondo-State. N igeri .

Dr. Zohairl.F. Rahemp
Department of Biology, Unive rsity of
Salahaddin , Ebi l, Kurdist n, Iraq

Dr. Kishor G. Patil,
P.G. Depar ment of Zoology,
Institute of Science, Nagpur, Indi

Dr RajusingG . Jadhao
Department of Zoology, Shri Shiv aji
Scienc e College, Amravai, I ndia

Dr.Muku nd M.Dh ore
Depar ment of Botany,
B.B. Arts, N.B. Comm. & B.P. Science
College, Digras ,MS India.

Dr. Sushant Punekar
Govt Autonomous P.G. College,
Chhindwara (M.P.) India

Dr. S.S.Nanwar e
Departmento f Zoology,
Yashawant College,Nan ded, India

Dr.Vishal Maat he
Department of Botany, NES, Science
Collge, Nanded, India

Dr.S angitaIn gole
Depatme nt of Envionmental
Science, ShriShivaji Science
Col ege, Amravati(M S) India

Dr. Uma Rani Agrawal
Department of Zo ology,
CMP Dege e College,
Universty of Allahabad, Allahabad,
India

Associate Editor

Dr. Mahdi Esmaeilzadeh
Department of Immunology,
Division of Human Genetics,
Avicenna Research Institute,
Mashhad University ofMedical Sciences.
Bu-Ali Sq. Mashhad, Iran.

Dr. Mumtaz Baig
P.G. Department of Zoology, G.V.I.Sc.,
Amravati. 444604(MS) India

Dr. Rajendra V. Tijare,
Department of Zoology,
Institute of Science, Nagpur, India

Dr. Dhanraj V. Tayade
Department of Zoology
G.S. Gawande College,
Umarkhed, MS India

Member of Editorial Board

Dr. Gayathri Narayan
Department of Zoology,
D.G. Ruparel College of Arts,
Science & Commerce,
Mahim (W), Mumbai 400 016.

Dr. Kumud Choudhary
Department of Zoology,
Shri R. R. Lahoti Science College,
Morshi, Amravati (MS) India.

Dr. Piyush Gupta
Department of Environmental
Chemistry, Science and Humanities
SRM university, Modinagar,
Ghaziabad, india

Dr. Tushar Wankhede
Depratmnet of Botany,
Shri Shivaji Science College,
Amravati, India

Dr. Sachin Tawade
Department of Botany,
D.B. College, Bhokar, Nanded, India

Prof. Aruna U. Kakade
Department of Environment,
Shri Shivaji Science College,
Amravati, India

RESEARCH ARTICLE

Distribution, physiologic races and reaction of wheat cultivars to virulent races of leaf rust (*Puccinia triticina* Eriks and Henn.) in south eastern zone of Tigray, Ethiopia

Tesfay Gebrekirstos Gebremariam[1], Getaneh Woldeab[2] and Thangavel Selvaraj[1]*

[1]Department of Plant Sciences, College of Agriculture and Veterinary Sciences, Ambo University, Ambo, Post Box No: 19, Ethiopia.
[2]Department of Pathology, Ambo Plant Protection Research Center (APPRC), Ambo, Ethiopia.
*Corresponding author: Email: tselvaraj_1956@yahoo.com | Contact : +251-913073294.

Manuscript details:

Received: 07.11.2015
Accepted: 16.01.2016
Published : 10.03.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Tesfay Gebrekirstos Gebremariam, Getaneh Woldeab and Thangavel Selvaraj (2016) Distribution, physiologic races and reaction of wheat cultivars to virulent races of leaf rust (*Puccinia triticina* Eriks and Henn.) in south eastern zone of Tigray, Ethiopia. *International J. of Life Sciences*, 4(1): 1-21.

Acknowledgements
This research was conducted in partial fulfillment of the M. Sc., degree at Ambo University by the first author. Funding was provided by the Ministry of Education.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

Leaf rust (*Puccinia triticina* Eriks and Henn.) is one of the most important foliar diseases of wheat (*Triticum aestivum* L.) in South eastern zone of Tigray in Ethiopia. Regular surveying, race identification and searching for resistant genes plays significant role to develop resistant varieties against leaf rust. Hence, this study was carried out to determine the distribution and intensity of wheat leaf rust to identify physiologic races of *P. triticina* and also to evaluate the seedling reaction of commonly grown wheat varieties to virulent races of leaf rust. The results of this study were based on leaf rust survey to compute the prevalence and intensity of the disease; race identification through inoculation of leaf rust populations, isolation, multiplication of mono pustules of the pathogen and determination of races by inoculating on leaf rust differential hosts; and evaluating ten wheat varieties to virulent and dominant races (TKTT, THTT and PHTT) at seedling stage in greenhouse. During the survey, a total of 108 farmers' wheat fields and experimental plots were assessed in five districts of South Eastern Tigray, of which 95 of the fields (88%) were affected with leaf rust. The overall mean incidence and severity of the disease were 48.4 and 18.2%, respectively. The highest intensity of leaf rust was recorded in Wukro wheat fields with incidence of 63.2% and severity of 37.3%. In contrast, the lowest incidence and severity of the disease were recorded in D/ Temben district with mean values of 7.4 and 4.1%, respectively. The characterization of 40 mono pustules of *P. triticina* was resulted for the identification of 22 races. Races PHTT and PHRT were predominant with frequencies of 20 and 15%, respectively, followed by THTT and FHRT with a frequency of 10% each. The remaining 18 races were confined to specific locations and detected once with a frequency of 2.5% each. The broadest virulence spectrum was recorded from TKTT race, making all *Lr* genes except *Lr* 9 was ineffective. About 81% of the *Lr* genes were ineffective to more than 55% of *P. triticina* isolates. High virulence was observed on *Lr*3, *Lr*10, *Lr* B and *Lr*18 with frequencies of 90, 95, 97.5 and 100%, respectively. However, *Lr* genes 9, 24 and 2a were found effective

to 100, 95 and 82.5 of the tested isolates, respectively. The variety evaluation revealed that, Mekelle-3, Mekelle-4, Picaflor, Dashin and local showed susceptible reactions to TKTT, THTT and PHTT races. Mekelle-1 and Mekelle-2 were susceptible to races TKTT and THTT, but resistant to PHTT. Digalu was only susceptible to TKTT race. Unlike bread wheat varieties, the durum varieties, Ude and Dembi were resistant to these races. The results of this study showed that most bread wheat varieties did not have adequate resistance for leaf rust. Hence, the gene pyramiding of *Lr*9, *Lr*24 and *Lr*2a has paramount importance as the additive effects of several genes offer the variety a wider base for leaf rust resistance.

Keywords: Bread wheat, Durum wheat, Leaf Rust, Isolates, *Lr* genes.

INTRODUCTION

Wheat (*Triticum* spp.) is one of the important leading cereal grain, where 40% of the world population uses as a staple food (Anonymous, 2007). It is grown on 255 million hectares worldwide from the equator to the latitudes of 60^0N and 44^0S and at altitude ranges from sea level to 3000 m. a. s. l. Approximately 600 million tons of grain is produced annually, roughly half of which is in developing countries (Aquino *et al*., 2002). Its popularity comes from the versatility of its use in the production of a wide range of food products, such as "Injera", breads, cakes, pastas, cookies etc (Pena, 2002). In addition, it has high nutritive value (>10% protein, 2.4% lipid, and 79% carbohydrates) and it accounts for about 20% of the caloric intake of the human diet (Gooding and Davies, 1997). Wheat is one of the major cereal crops grown in the highlands of Ethiopia; particularly, the Tigray region in Ethiopia is regarded as the second largest wheat producer in Sub-Saharan Africa (White *et al*., 2001). The major wheat production areas are Western Harerghe, Illubabur, Bale, Arsi, Shewa, Sidamo, Tigray, Northern Gondar and Gojam zones (Bekele, 2000). The area under wheat production is estimated to be about 1.5 million hectare and ranks third after maize and teff (CSA, 2009). Wheat is cultivated in a wide altitude range from 1500-3000 m. a. s. l. However, the most suitable agro-ecological zones for wheat production fall between 1900- 2700 m. a. s. l (Hailu, 1991). This crop is used as a staple food for about 36% of the Ethiopian population (CIMMYT, 2005).

In Tigray region, wheat has been selected as one of the target crops in the strategic goal of attaining regional food self-sufficiency. In this region, wheat covers over 0.1 million hectare with total production of 1.93 million quintals annually (CSA, 2011). South eastern zone of Tigray is one of the major wheat growing areas and recognized as wheat belt in the region. It is a pillar crop and covers an area of 49,244 hectares in south eastern zone of the region (BoARD, 2005). Although, the area cultivated under wheat has been increased in the last few years, the production and productivity of the crop in Ethiopia in general and Tigray region in particular is still very low. The national average yield is estimated to 1.7 tons/ha (CSA, 2009). This is by far below the world's average yield which is about 3.3 tons/ha (Curtis *et al*., 2002). The low productivity of the crop is attributed to number of factors including biotic (diseases, insects and weeds), abiotic (drought, acidity, alkaline, extreme temperatures, depleted soil fertility and snow) and low adoption of new agricultural technologies (Ayele *et al*., 2008). Among these factors, plant diseases are the major biotic constraints of wheat production in the world including Ethiopia. The rusts are the most destructive diseases of wheat worldwide (Singh *et al*., 2008). The three ruts; stem (*P. graminis f. sp. tritici*), leaf (*P. triticina*) and stripe rust (*P. striiformis f. sp. tritici)* are foliar and stem diseases that causes significant reduction in yield and quality in different regions of the world (Kuraparthy *et al*., 2007). Wheat rusts have reported as devastating, having the ability to destroy entire susceptible wheat crops in a matter of weeks and resulting in large economical losses (Marsalis and Goldberg, 2006).

Wheat leaf rust, also known as brown rust, caused by the fungal pathogen, *Puccinia triticina* Eriks and Henn., possibly the most widespread of the wheat rusts and occurs in most wheat growing areas of the world (Kolmer, 2005). The wide range adaptability helps this fungus to co-exist with wheat wherever it is grown

(Winzeler *et al.,* 2000). In Ethiopia, wheat leaf rust is one of the most important diseases of wheat, and its recurrent outbreaks have threatened wheat production in the country (Bedabo, 2002). The high virulence diversity and evolution rate of the pathogen makes a lot of wheat cultivars at risk in our country. For instance, out of the 26 wheat cultivars released in the period 1970 to 1993, only three retained their resistance to leaf rust (Geleta and Tanner, 1995). During 2007-2009 cropping seasons, incidence of 30.2% was recorded for leaf rust in Oromia, Amhara, SNNPP and Tigray regions. Prevalence of leaf rust for the above mentioned locations was 53.3%. In 1976, leaf rust was recorded in all wheat growing regions of the country with severity range of 40-50% in local cultivar, while 100% on susceptible checks at Kulumsa (Madumarov, 1977). In Tigray region, wheat diseases survey was also made in 1994 and 1995 cropping seasons and identified eight fungal diseases (MARC, 1998). Leaf rust and yellow rust were found to be the most important diseases affecting wheat production in the region in general and South eastern zone in particular (MARC, 1998). In Ethiopia, yield loss due to leaf rust reached 75% in susceptible wheat varieties at hot spot areas (Mengistu *et al*., 1991). This yield loss is attributed due to infection of the flag leaf, which is thought to be responsible for greater than 70% of grain filling (Feyissa *et al.*, 2005).

Use of host resistance is the most economical and environmentally friendly method of controlling wheat leaf rust (Afzal *et al.,* 2009). In Ethiopia, wheat production is characterized by high biodiversity in crops and low input systems, and the control of rust diseases largely relies on genetic resistance (Sewalem *et al.,* 2008). However, host resistance may not always be readily available for use against leaf rust, and it requires regular surveying, race identification and continuous search for new sources of resistant genes in the cultivated and wild forms of wheat (Kuraparthy *et al.,* 2007). In Ethiopia, many studies on virulence and race identification of wheat leaf rust were carried out in different times. For instance, according to information obtained from SPL, 57 leaf rust races were identified, among these, race1, 53, 58, 61, 62, and 141 were predominant (Solmatin and Teman, 1985). Similarly, 23 races of wheat leaf rust were analyzed from 31 *P. triticina* isolates collected from Central and South eastern part of Ethiopia (Sewalem *et al*., 2008). During this time, virulence was reported on *Lr* genes *Lr11, Lr12, Lr13, Lr22, Lr33, Lr34, Lr35, LrB* and *LrTc*, whereas no virulent races were identified for genes *Lr9, Lr19, Lr24, Lr26, Lr29, Lr38,* and *LrW*. Mideksa (2011) also reported that, 19 races from 46 isolates of *P. triticina* were identified from two districts of West Shewa zone. In his study, *Lr* genes namely, *Lr2a, Lr2c, Lr3, Lr9, Lr16, Lr24, Lr3ka* and *Lr30* were effective to most of wheat leaf rust isolates. However, this work was covered mainly Central, West Showa and Southeastern parts of Ethiopia. Other parts of the country like Tigray region in general and Southeastern zone in particular, do not have information on the distribution, physiologic races and response of wheat varieties to leaf rust populations. Therefore, this study was carried out to determine the distribution and intensity of wheat leaf rust to identify the physiologic races of *Puccinia triticina* in South eastern zone of Tigray, Ethiopia and also to evaluate the seedling reaction of commonly grown wheat varieties to virulent races of leaf rust in green house conditions.

MATERIALS AND METHODS

Description of the study area

Tigray region is comprised of diverse topographic features including, about 53% lowland (1400-1800 m. a. s. l), 39% midland (1800-2400 m. a. s. l) and 8% highland (2400-3400 m. a. s. l) and the region is also classified in to three agro ecological zones of 67% dry, 24% moist and 9 % wet (BFED, 2007). The climate of the region is generally sub tropical with an extended dry period of nine to ten months and maximum effective rainy season of 50 to 70 days. The mean annual rain fall ranges from 500-1000 mm (ENMA, 2013). The rainfall pattern is predominantly uni-modal (June to September). Considering rainfall and temperature, more than 90% of the region is categorized as semiarid. The remaining areas of the region can be categorized as dry sub-moist and arid. The region is divided into six zones: Central, West, Northwest, East, Southeast and Southern. South eastern Zone of the region includes five districts viz; Enderta, H/wejirat, S/ Samre, Degua Temben and Wukro (Fig. 1). The major area of this zone is mid highland and highland with temperature range of 10-25.5^{o}C and the annual average rainfall fluctuates between 500-907 mm. This zone is located at 39^{0} 48'East longitude and 13°5'North latitude at an elevation of 1970- 2589 m. a. s. l.

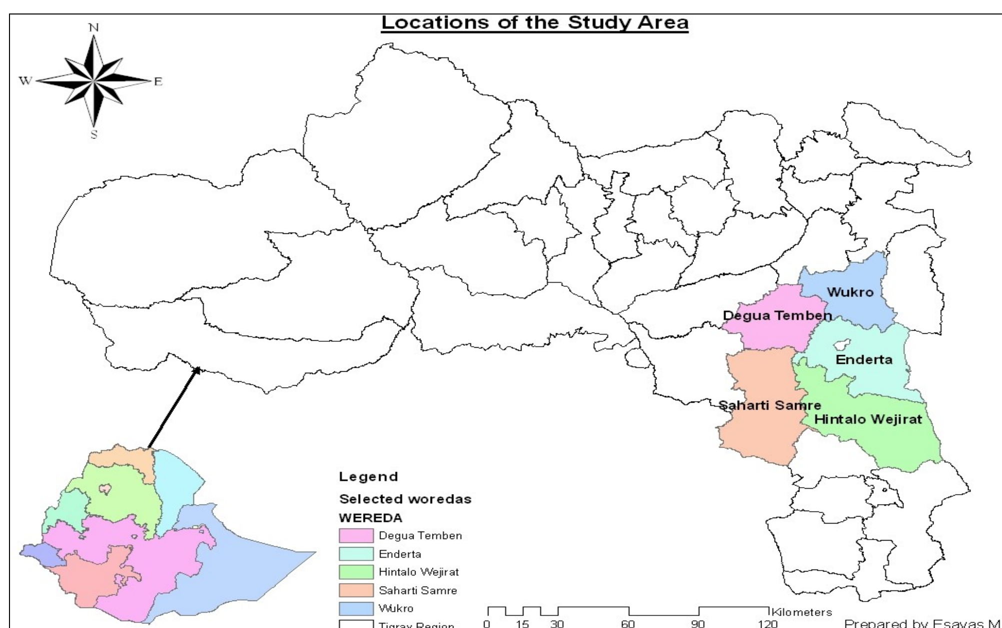


Fig. 1. Map of the five surveyed districts of the South eastern zone of Tigray, Ethiopia.

Survey of leaf rust in south eastern zone of Tigray

Field survey of leaf rust was carried out in the major wheat growing areas of south eastern zone of Tigray in 2013 main growing season. Private farms in five districts (Wukro, H/wejirat, S/samre, D/Temben and Enderta) and experimental plots of MARC were included in the assessment. In each district, the localities were selected based on the data of dominance of wheat production obtained from the BoARD of the respective districts. A total of 10 localities, three from H/wejirat, two each from Wukro, Enderta and S/Samre and one from D/Temben were assessed for wheat leaf rust infection. Wheat fields along the main and accessible road sides were inspected at 5-10 kilometer intervals. A total of 108 wheat fields were surveyed at critical growth stage of the crop (flag leaf stage) during which leaf rust reached its maximum severity level (Seck *et al*., 1985). Leaf rust assessment was made along the two diagonals (in ‘’X’’ fashion) of the field at five points using 0.5m^2 quadrant. In each field, plants within the quadrant were counted as diseased/ infected and healthy / non- infected and the incidence, severity and prevalence of wheat leaf rust were calculated as follows:

$$\text{Incidence (\%)} = \frac{\text{Number of diseased plants X 100}}{\text{Total number of plants assessed}}$$

Severity (%) =Leaf area infected (Peterson *et al*., 1948)

$$\text{Prevalence (\%)} = \frac{\text{Number of leaf rust affected fields X 100}}{\text{Total number of fields assessed}}$$

The severity of leaf rust was examined visually on whole plants within the quadrants and recorded as the percentage of plant parts or tissue affected (percentage of rust infection of the plant), and plant response (type of infection) using modified Cobb's scoring scale of rust disease under field conditions (Peterson *et al.,* 1948) (Fig. 2). The host response to infection in the field scored using 'R' to indicate resistance or miniature uredinia; ‘MR’ indicate moderate resistance, expressed as small uredinia; 'MS' to indicate moderately susceptible, expressed as moderate sized uredinia somewhat smaller than the fully compatible type and 'S' to indicate full susceptibility. Moreover, data on geographic information (latitude, longitude and altitude) of each field was recorded using GPS (e Trex Legend GPS system, Garmin). The GPS data were later used to plot surveyed fields on a map using the computer program Arc view 3.0 (ESRI). In addition, for each surveyed wheat field, information like wheat type, variety and growth stage of the crop was collected. Growth stage of the crop was recorded based on Zadoks *et al.,* (1974). The prevalence, incidence and severity data were analyzed by using descriptive statistical analysis (means) over districts, localities, varieties, altitudes and crop growth stages.

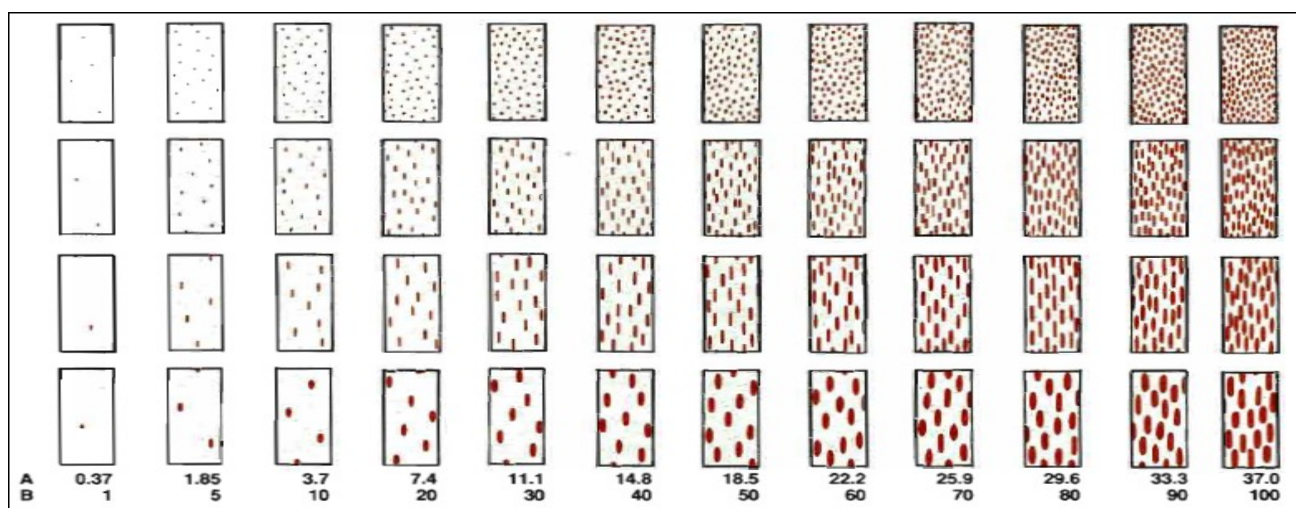


Fig. 2 The modified Cobb Scale: (A) Actual percentage occupied by rust uredinial; (B) Rust severities of the modified Cobb Scale after Peterson *et al*., (1948).

Collection of wheat leaf rust samples

Leaf rust infected samples (five samples per locality) were collected from wheat fields and experimental plots of MARC (Fig 3 a & b) Infected leaves were cut from the mother plant using scissors and placed in an envelope. Samples collected in an envelope were labeled with all necessary information including name of the region, zone, district, variety, GPS data and date of collection. Samples were kept in refrigerator until the survey in all districts was finalized. Then after, samples were preserved in ice box and transported to APPRC laboratory for race analysis and variety evaluation studies. The samples were kept in the refrigerator at 4ºc until used for the virulence analysis.

Isolation and multiplication of *P. triticina* inoculum

The inoculum was increased and maintained on universally rust susceptible variety "Morocco" which does not carry any known leaf rust resistant gene (Roelfs *et al*., 1992). Six seedlings of "Morocco" were raised in suitable 8 cm diameter clay pots containing sterilized soil, sand and manure in a ratio of 2:1:1 mixture, respectively. Seven day-old seedlings or when the primary leaves were fully expanded and the second leaves beginning to grow, the leaves were rubbed gently with clean (dis-infected with 97% of alcohol) moistened (with distilled water) fingers to remove the waxy layer from the surfaces of the leaves (Fig. 3 c & d). Green house inoculations were done using the methods and procedures developed by Stakman *et al*. (1962). Bulked spores from the leaf rust infected samples were collected with scalpels and transferred on to a watch glass which contain distilled water to make spore suspension, and then it was rubbed on seedlings of Morocco with clean moistened fingers. The plants were then moistened with fine droplets of distilled water produced with an atomizer and incubated in dew chamber for 24 hours at 18-20ºC with 90% RH and seedlings were allowed to dry gradually. Then, the seedlings were transferred from the dew chamber to glass compartments where conditions were regulated at 12 hours photoperiod, at temperature of 18-25ºC and RH of 60-70%. The remaining spore samples were kept in refrigerator at 4ºC and were used to substitute samples which failed to produce infection on the universally susceptible variety.

After seven days of inoculation, when the flecks/chlorosis were clearly visible, leaves containing single flecks were selected from the base of the leaves and the remaining leaves within the pots were removed using scissors. Only 2-3 leaves which contains mono pustule were covered separately with cellophane bags (145 X235 mm) and tied up at the base with a rubber band to avoid cross contamination (Fetch and Dunsmore, 2004). After 12-14 days of inoculation, when the mono pustule was well developed, each mono pustule was collected in a test tubes separately using vacuum pump A suspension, prepared by mixing mono pustule urediospores with light weight mineral oil (SolTrol 130), was inoculated on seven day- old seedlings of 'Morocco' for multiplication purpose. After inoculation, seedlings were placed in dew chamber for 24 hours at 18-22ºC

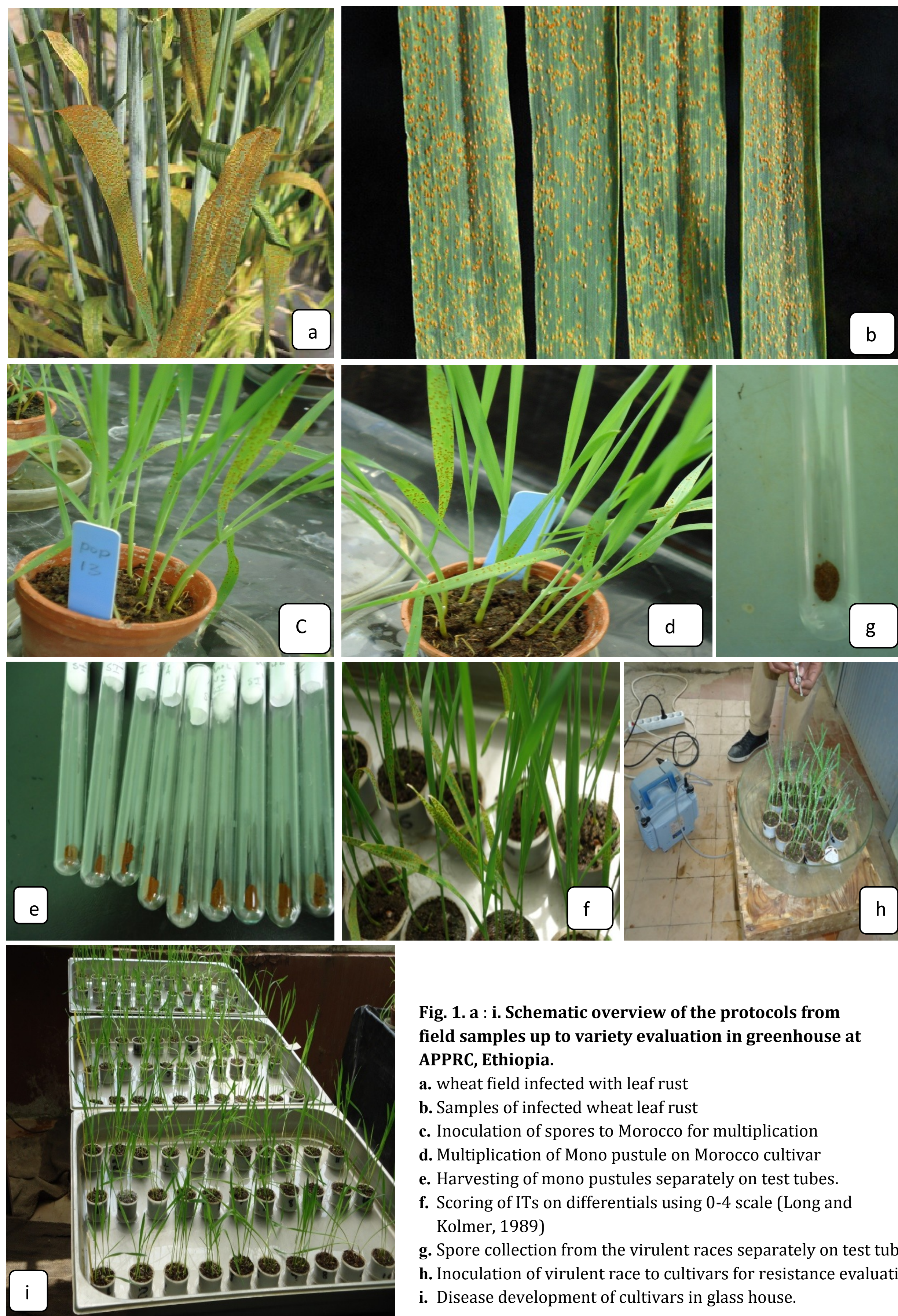


Fig. 1. a : **i. Schematic overview of the protocols from field samples up to variety evaluation in greenhouse at APPRC, Ethiopia.**

a. wheat field infected with leaf rust
b. Samples of infected wheat leaf rust
c. Inoculation of spores to Morocco for multiplication
d. Multiplication of Mono pustule on Morocco cultivar
e. Harvesting of mono pustules separately on test tubes.
f. Scoring of ITs on differentials using 0-4 scale (Long and Kolmer, 1989)
g. Spore collection from the virulent races separately on test tube
h. Inoculation of virulent race to cultivars for resistance evaluation.
i. Disease development of cultivars in glass house.

Table 1: List of sixteen wheat leaf rust differential hosts with their corresponding *Lr genes* and pedigree

Differential lines	*Lr* genes	Pedigree	Differential lines	*Lr* genes	Pedigree
RL6003	Lr1	TC*6/Centenario	RL6007	Lr3ka	TC*6/Klein Aniversario
RL6016	Lr2a	TC*6/Webster	RL6053	Lr11	TC*2 Hussar
RL6047	Lr2c	TC*6/Loros	RL6008	Lr17	Klein Lucero/ 6*TC
RL6002	Lr3	TC*6/Democrat	RL60 49	Lr30	TC*6/Terenzio
RL6010	Lr9	Transfer /6*TC	RL6051	LrB	TC*6/Carina
RL6005	Lr16	TC*6/Exchange	RL6004	Lr10	TC*6/Exchange
RL6064	Lr24	TC*6/Agent	RL6013	Lr14a	Selkirk /6*TC
RL60 78	Lr26	TC*6/St-1-25	RL6009	Lr18	TC*7/Africa43

Sources: Long and Kolmer, 1989

Table 2: Description of infection types to classifying the reactions of leaf rust on wheat seedlings in greenhouse

Infection types	Host response	Symptoms
0	Immune	No visible uredia
;	Highly resistant	Hypersensitive fleck
1	Resistant	Small uredia with necrosis
2	Resistant to moderately resistant	Small to medium size of uredia surrounded by necrosis or chlorosis
X	Mesothetic or Heterogeneous	A range of infection type from resistant to susceptible scattered randomly on a single leaf caused by single isolate
3	Moderately resistant to Moderately Susceptible	Medium size uredia with or without chlorosis
4	Highly susceptible	Large uredia without chlorosis
	Modified characters	
=	Low uredia	Uredia much smaller than the typical and at the lower limit of the infection type
-	Smaller uredia	Uredia smaller than normal
+	Large uredia	Uredia larger than normal
++	Largest uredia	Uredia much larger than typical and at the upper limit for the infection type

Source: Long and Kolmer, 1989

and with RH of 90%. Then after, seedlings were transferred to growth chamber where conditions were regulated at 12 hours photoperiod, 18-25°C and RH of 60-70% following the procedures mentioned earlier. After 12-14 days of inoculation, the spores from each mono pustule/ isolate were collected in separate test tubes (Fig 3 e) and stored at 4°C until they were inoculated on the differential hosts. This procedure was repeated until sufficient amount of spores are produced to inoculate the set of wheat leaf rust differential host (Table 1). By doing this a total of 40 isolates/mono pustules were developed from 40 wheat leaf rust samples. A pictorial schematic overview of the general protocol used for race analysis in APPRC green house, Ethiopia (Fig. 3 a - i).

Inoculation of P. triticina isolates to wheat leaf rust differential hosts

Six seeds each from 16 differential hosts and "Morocco" were grown in 3cm diameter pots containing soil, sand and compost at a ratio of 2:1:1 respectively. The susceptible variety Morocco was used as a check. The single pustule derived spores (approximately 3-5 mg of spores per ml of liquid suspension) was suspended in distilled water and sprayed onto seven-day-old seedlings using atomizers. After inoculation, plants were moistened with fine droplets of distilled water produced with an atomizer and placed in dew chamber for 24 hours at 18-22°C and RH of 90%. Up on removal from the dew chamber, plants were placed separately in the growth chamber

to avoid contamination. Greenhouse temperatures were maintained between 18-25°C.

Leaf rust assessment on differential hosts

Twelve days after inoculation, the infection types were scored for each isolate using the 0-4 scoring scale of Long and Kolmer (1989) (Table 2). Infection types were grouped in to two, where, Low (Resistance) = incompatibility (infection types: 0 to 2) and High (Susceptible) = compatibility (infection type: 3 to 4). Avirulence and virulence of cultures were determined by low (L) and high (H) infection types, respectively.

Designation of races of Puccinia triticina

Race designations were assigned as described by Long and Kolmer (1989). Race designation was done by grouping the sixteen differential hosts into four sets in the following order: (i) Lr1, Lr2a, Lr2c, Lr3; (ii) Lr9, Lr16, Lr24, Lr26; (iii) Lr3ka, Lr11, Lr17, Lr30 and (iv) LrB, Lr10, Lr14a, Lr18 (Table 3).

Each isolate was assigned a four letter race code based on its reaction on the differential hosts (Long and Kolmer, 1989). For instance, low infection type (L) on the four hosts in a set is assigned with the letter 'B', while high infection type (H) on the four hosts is assigned with a letter 'T'. Hence, if an isolate produces low infection type (resistant reaction) on the 16 differential hosts, the race will be assigned with a four letters race code 'BBBB'. In the same way, an isolate which produces a high infection type (susceptible reaction) on the 16 wheat differential hosts have a race code 'TTTT' (Table 3). If an isolate produces a low infection type on Lr9 but a high infection type on the remaining 15 differential hosts, the race will be designated as TKTT.

Response of wheat cultivars to leaf rust races at seedling stage in green house.

The isolates of prevalent and virulent leaf rust races identified from the southeastern zone of Tigray were multiplied on the universally susceptible variety Morocco and collected in separate test tubes to inoculate wheat cultivars (Fig 3 f - i). Ten wheat varieties (Table 4) mainly cultivated in Tigray region were evaluated against the virulent race TKTT and dominant leaf rust races THTT and PHTT. Six seeds of each cultivar and Morocco were sown in 3 cm diameter plastic pots separately. Seven day old seedlings (when the first leaf is fully expanded and the second leaf is just emerged) were inoculated with spores of the mentioned races and incubated (Fig. 3 h). Varieties were arranged in complete randomized design (CRD) and replicated three times (Fig. 3 i). Data on infection types was recorded 12 days after inoculation using standard disease scoring scale 0- 4 (Long and Kolmer, 1989) (Table 2) and analyzed by using descriptive statistical analysis (means).

Table 3: Nomenclature of *Puccinia triticina* races on 16 differential hosts in ordered sets of four

Pt code	Host set	Infection type (ITs) produced on differential *Lr* lines			
	Host set 1	1	2a	2c	3
	Host set 2	9	16	24	26
	Host set 3	3ka	11	17	30
	Host set 4	B	10	14a	18
B		L	L	L	L
C		L	L	L	H
D		L	L	H	L
F		L	L	H	H
G		L	H	L	L
H		L	H	L	H
J		L	H	H	L
K		L	H	H	H
L		H	L	L	L
M		H	L	L	H
N		H	L	H	L
P		H	L	H	H
Q		H	H	L	L
R		H	H	L	H
S		H	H	H	L
T		H	H	H	H

Source: Long and Kolmer, 1989

Table 4: List of wheat varieties for evaluation of resistance against leaf rust races at their seedling stage in the greenhouse

S.N	Variety	Wheat type	Pedigree
1	Mekelle 1	Bread wheat	PARULA[2800]
2	Mekelle 2	Bread wheat	HINDI62/BOBWHITE/CPAN2099
3	Mekelle 3	Bread wheat	NA
4	Mekelle 4	Bread wheat	NA
5	Picaflor	Bread wheat	CHIL/PRL
6	Digalu	Bread wheat	SHA7/KAUZ
7	Dashin	Bread wheat	VEE 17/KVZ/BUHO"S"//KAL/BB
8	Ude	Durum wheat	CHEN / ALTAR- 84// JO69 CD 95294-9M- 030Y-040 PAP-2Y-OB
9	Dembi	Durum wheat	VZ466/61-130XLD SX GII"S" CM9605
10	Local cultivar	Bread wheat	NA
11	Morocco (Sus. check)	Bread wheat	NA

NA= not available

RESULTS AND DISCUSSION

Survey of wheat leaf rust in south eastern zone of Tigray

Plant disease assessment is to measure the amount of disease present in terms of incidence and severity of the individual plants. Keeping this in mind, survey of leaf rust was carried out from early to mid of September in 2013 in five districts of Southeastern Zone of Tigray. During the survey, 108 wheat fields were assessed for leaf rust distribution and intensity. Whenever disease assessments are made, growth stage of the plants is essential for meaningful comparisons between varieties, locations and years (Stubbs *et al*., 1986). In view of this, 2.8% of the wheat crop was at tillering to booting, 50% at heading to flowering and 47.2% at milk to dough growth stages. Leaf rust was observed in 2(66.7%), 45(83.3%) and 47(92.2%) of the 3, 54 and 51 wheat fields inspected during tillering to booting, heading to flowering and milk to dough growth stages of the crop respectively.
As far as disease intensity was concerned, leaf rust was found more important at heading to flowering and followed by milk to dough growth stages of the crop (Table 5). During heading to flowering growth stages, mean incidence of 67.2% and severity of 26.9% were recorded. The disease also caused high infection at milk to dough growth stages, with mean incidence and severity of 44.2% and 12.7%, respectively. Earlier study also indicated that, highest intensity of wheat leaf rust was recorded at heading to flowering growth stages of the crop (Mideksa, 2011).

Conversely, the lowest incidence and severity of leaf rust was recorded at tillering to booting growth stages of the crop with mean values 21.2% and 6.7%, respectively. This might be resulted from the fact that, the flag leaf which is the critical growth stage of the crop during which leaf rust reached its maximum severity level (Seck *et al.,* 1985), was not fully develop at tillering to booting growth stages as compared to heading to flowering and milk to dough growth stages of the crop . On top of this, damage is minimal during tillering due to the onset of colder temperatures that are likely to eliminate or reduce reproduction and spread of rusts.

Distribution and intensity of wheat leaf rust across districts

The disease was more prevalent at H/wejirat and Wukro districts with prevalence of 100% and 96.7% respectively, while districts of Enderta and S/samre showed similar distribution of wheat leaf rust, 85% each. In contrast, the lowest prevalence (45.5%) of leaf rust was registered at D/ Temben district. As a whole, wheat leaf rust was observed on 88% of the 108 wheat fields inspected in southeastern zone of Tigray (Table 6). This indicated that, the pathogen was widely distributed across the districts of the study area. As far as leaf rust intensity was concerned, different mean incidences and severities were recorded in all districts. The highest mean incidence of the disease was noted in Wukro district with range of 0-100% and a mean value of 63.2% and followed by S/samre, with range of 0-100% and mean incidence of 60%. Districts of Enderta and H/wejirat also showed a considerable level of leaf rust incidence with mean values of 57.9% and 53.3%, respectively (Table 6). However, the lowest incidence of the disease (7.4%) was registered in D/Temben district. The overall mean incidence for the surveyed districts of southeastern zone of Tigray reached 48.4%.

Table 6: Prevalence and intensity of wheat leaf rust across districts in 2013 main cropping season

Districts	Altitude range (masl)	Number of fields inspected	Number of fields infected	Prevalence (%)	Incidence (%)		Severity (%)	
					Range	Mean	Range	Mean
H/wejirat	1966-2198	27	27	100	5-100	53.3	1-75	14.1
Wukro	1927-2399	30	29	96.7	0-100	63.2	0-95	37.3
S/Samre	1961-2339	20	17	85	0-100	60	0-85	26.1
Enderta	1912-2292	20	17	85	0-100	57.9	0-25	9.5
D/Temben	2431-2654	11	5	45.5	0-24	7.4	0-10	4.1
Total /Mean	1912-2654	108	95	88	0-100	48.4	0-95	18.2

Likewise, leaf rust severity showed similar trend as that of incidence in Wukro, S/samre and D/Temben districts. The highest severity was recorded in Wukro district with a range of 0-95% and mean value of 37.3%. This was followed by S/samre district, with range of 0-85% and mean severity of 26.1%. The highest severity of 37.3% was recorded where the highest incidence of 63.2% was registered at Wukro district. In a similar trend, the lowest mean severity of 4.1% was recorded where the lowest mean incidence of 7.4% was registered at D/Temben district. Unlike the three districts mentioned above, leaf rust severity did not show similar trend as that of incidence in Enderta and H/wejirat districts. Enderta and H/wejirat were ranked third and fourth in terms of incidence with mean values of 57.9 and 53.3%, respectively. Conversely, these districts were ranked as fourth and third in terms of severity with mean values of 9.5 and 14.1% following the same order. This indicated that, incidence did not show direct relationship to the severity of leaf rust in these districts. This might be associated with fact that, plants were counted as diseased whether they were exhibiting a single pustule or hundreds of pustules during recording incidence. But, in case of severity, plants with hundreds of pustules were considered as more diseased than plants with single pustule. Hence, incidence may have little relationship to the severity of the disease under such circumstance. The overall mean severity for the five districts of southeastern zone of Tigray reached 18.2% (Table 6).

Generally, leaf rust was found more important at Wukro, S/samre, H/wejirat, and Enderta districts. The high level of leaf rust intensity in these districts might be the cultivation of local cultivar or “Shahan” which is susceptible to wheat rusts in general and leaf rust in particular. This cultivar covered about 53.3, 55.6, 57.9 and 65% of the wheat area in Wukro, H/wejirat, Enderta and S/samre districts respectively (BoARD, 2005). Moreover, the cultivation of wheat in some areas of these districts during offseason could have played a significant role in rehabilitating the population of wheat leaf rust. Hence, these varieties could act as a green bridge to carryover the disease from offseason to main season. Earlier study also indicated that the presence of two overlapping seasons for growing wheat (meher and belg seasons) helps in the buildup of inoculum in one season and transferred to the other season as a source of primary inoculum, facilitating the availability of inoculum year after year in the country (Serbessa, 2003). However, the low prevalence and intensity of leaf rust in D/Temben district might be resulted from low temperature occurred during the season. In this district, the temperature was less than 10°C (ENMA, 2013) which is below the optimum level of temperature. This low temperature likely eliminates or reduces the reproduction and spread of the rust. Earlier studies also confirmed that, at 10°C, infection developed very slowly and restricted in size (Dyck and Johnson, 1983).

Distribution and intensity of wheat leaf rust across localities

The ‘within district’ comparison indicated that, highest prevalence (100%) of the diseases was recorded in all localities of H/wejirat. This implied that, the population of leaf rust was uniformly distributed across wheat fields of this district. However, these localities were infected with different levels of incidences and severities of leaf rust. The disease was more important in Adigudom, Hiwane and Freweyni localities with mean incidences of 83.8%, 65.3% and 10.9% in that order. The range of incidences in these localities also showed the same order as that of mean incidence. Similarly, the severity of this disease in these localities showed similar trend as that of incidences. The highest mean severity of 19% was recorded in Adigudom, followed by Hiwane with mean value of 17.6%. Contrary to this, the lowest severity of

Table 7. Prevalence and intensity of wheat leaf rust across localities of Southeastern Zone of Tigray, in 2013 cropping season

District	Kebele (locality)	Altitude range (masl)	No. fields inspected	No. fields infected	Prevalence (%)	Incidence (%)		Severity (%)	
						Range	Mean	Range	Mean
H/wejirat	Hiwane	1966-2066	10	10	100	19-95.5	65.3	1-40	17.6
	Adigudom	2048-2198	10	10	100	42.5-100	83.8	5-75	19
Wukro	Freweyni	2012-2195	7	7	100	5-30	10.9	5-10	5.7
	Genfel	1935-2399	16	15	93.8	0-100	63.6	0-95	28.4
	Adimekel	1927-2091	14	14	100	5-100	62.7	1-95	46.1
S/Samre	Degen	2001-2339	11	8	72.7	0-82	36.8	0-30	10
	Mytekli	1961-2123	9	9	100	20-100	83.2	5-85	42.2
Enderta	Quiha	2022-2292	10	8	80	0-100	65.1	0-20	8.8
	Illala	1912-2225	10	9	90	0-92.9	50.7	0-25	10.2
D/Temben	D/Temben	2431-2654	11	5	45.5	0-24	7.4	0-10	4.1
Total/mean	10	1912-2654	108	95	88	0-100	53	0-95	19.2

Table 8. Prevalence and intensity of leaf rust in different agro ecologies of Southeastern zone of Tigray in 2013 cropping season.

Altitude range (masl)	Number of Inspected field	Number of Infected fields	Prevalence (%)	Incidence (%)		Severity (%)	
				Range	Mean	Range	Mean
1801-2300	91	84	92.3	5-100	59.9	Trace- 95	21.4
2300 -2654	17	11	64.7	1-100	29	Trace-15	5.6
Total/Mean	108	95	78.5	1-100	44.5	Trcea-95	13.5

Source: IFPRI, 2006

wheat leaf rust was recorded in Freweyni with mean value of 5.7%. In Wukro district, the disease was also highly distributed with prevalence of 93.8% in Genfel and 100% in Adimeskel. These localities showed similar mean incidences of leaf rust with 63.6% in Genfel and 62.7% in Adimeskel wheat fields. However, they showed differences in severities of the disease, in which the highest mean severity of 46.1% recorded in Adimeskel and 28.4% in Genfel wheat fields. The low incidences and severities in these localities resulted from low or free infection scored from durum wheat varieties, Ude and Dembi grown in both localities for seed multiplication. The prevalence and intensity of the disease were different across localities of Degen and Mytekli in S/samre district. Leaf rust was uniformly distributed in Mytekli with prevalence of 100%, while 72.7% was recorded in Degen wheat fields. The highest mean incidence (83.2%) and severity (42.2%) were recorded where the highest prevalence (100%) was noted in Mytekli. In the same way, the lowest mean incidence (36.8%) and severity (10%) were recorded where the lowest prevalence (72.7%) was noted in Degen. This indicated that, the distribution of the disease in these localities sowed the same trend as that of incidence and severity.

In contrast, less similarity trend among prevalence, incidence and severity of leaf rust was observed in Enderta localities. The highest prevalence of 90% and mean severity of 10.2% were recorded in Illala, while prevalence of 80% and mean severity of 8.8% were recorded in Quiha. But, a higher mean incidence of 65.1% was scored in Quiha, followed by Illala with 50.7%. Comparing with the listed localities, the lowest prevalence and intensity of leaf rust was recorded in D/Temben wheat fields. In this study area, the disease was distributed intermediately with prevalence of 45.5% and mean vales of 7.4% and 4.1% incidence and severity respectively (Table 7).

Distribution and intensity of wheat leaf rust across altitude ranges

Of 108 wheat fields inspected, approximately 84% of the fields were found with altitude range of 1801-2300 m. a. s. l, while the remaining 16% were found between 2300-2654 m. a. s. l. The highest prevalence of the disease was recorded at altitude range between 1801-2300 m. a. s. l. Out of 91 wheat fields inspected in this altitude range, the disease was found in 84 wheat fields. Similarly, the highest incidence and severity of wheat leaf rust were recorded at this altitude range of 1801-2300 m. a. s. l with range and mean values of 5-

100% and 59.9% and Trace-95% and 21.4% respectively. However, the distribution and intensity of wheat leaf rust reduced at higher altitudes. Relatively low prevalence (64.7%) of leaf rust was recorded at altitude range of 2300-2652 m. a. s. l. In the same way, the range and mean values of incidence and severity were reduced to 1-100% and 29% and Trace-15% and 5.6%, respectively. The overall mean incidence and severity of wheat leaf rust for the midland (1801-2300 m. a. s. l) and highland (2300-2654 m. a. s. l) of the study area reached 44.5 and 13.5%, respectively (Table 8). In general, though, the disease was more important in midland areas, it was also distributed in the highland of the study area with considerable amount of intensity and prevalence. This might be associated with the fact that, wheat leaf rust occurs wherever wheat is grown and it is the most widely distributed of all cereal rusts (Knott, 1989). Moreover, the adaptability of leaf rust to different climates play a significant role for the widely distribution of the pathogen (Roelfs and Singh, 1992). This result is also similar with the findings of Dagnachew (1967). He reported that, leaf rust is endemic at different altitudes of wheat growing regions of Ethiopia.

Prevalence and intensity of wheat leaf rust by wheat type and variety

Tesfaye and Getachew (1991) reported that tetraploid wheat (durum) species cultivated in Showa, Gojam, Gonder, Wello and Tigray occupied 60% of the total wheat area in the country. However, recent studies that were conducted in the above mentioned regions and in the country as whole (Belaynesh, 2010) as well as this survey showed hexaploid wheat (bread) was dominating over the tetraploid wheat species. In view of this, about 95% of wheat fields in the study area were covered by bread wheat varieties. The local cultivar (locally named as Shahan) dominated the bread wheat varieties and covered 56.5% of wheat field in the study area. This was followed by Picaflor, Dashen and Mekelle-1 which comprised 16.7%, 6.5% and 5.5%, respectively. On the other hand, durum wheat varieties, Ude and Dembi covered only 4.5% of wheat fields in southeastern zone of Tigray.

During the survey, the prevalence and intensity of leaf rust varied between durum and bread wheat varieties (Table 9). Though, durum wheat varieties, Ude and Dembi showed prevalence of 100%, the intensity of the disease was lower on these varieties compared to Dashen, Digalu, Picaflor, Mekelle-3, Mekell-1 and local cultivar (Shahan). The range incidence and severity of leaf rust in durum varieties varied between 0-10 percent each respectively. The lowest mean incidence (2.5%) and severity (2.5%) of leaf rust was recorded on variety Dembi and followed by Ude, with mean incidence of 5% and severity of 3.8% across the three fields. The low intensity in these varieties was resulted from their resistant response recorded in all wheat fields of the study area. This finding is in agreement with previous report which stated that most of the commercial durum wheat cultivars exhibited stable resistance to wheat rusts across seasons in hot spot areas of Ethiopia and they could be exploited in wheat breeding programs (Efrem *et al.,* 1995). In contrast, the highest intensity of leaf rust was recorded in bread wheat varieties at different levels of incidences and severities. The prevalence and intensities of leaf rust in Mekelle varieties were lower as compared to Dashen, Digalu, Picaflor and Local cultivar. The prevalence of leaf rust in these varieties ranged between 50 -100% of the fields cultivated. The leaf rust incidences and severities in these varieties varied between 0-19.1%

Table 9. Prevalence and intensity of leaf rust on varieties grown in south eastern zone of Tigray in 2013 cropping season.

Varieties	Altitude range (m.a.s.l)	Number of fields Inspected	Number of fields infected	prevalence (%)	Incidence (%)		Severity (%)	
					Range	mean	Range	Mean
Mekelle- 1	1980-2142	6	4	66.7	5-19.1	5.9	1-5	5 R, MR
Mekelle -2	1970-2155	4	2	50	0-15	7.5	0-5	2.5R, MR
Mekelle- 3	1975-2165	3	2	66.7	0-15	7.5	0-10	5R,MR
Mekelle- 4	2012-2178	2	2	100	0-5	2.5	0-5	2.5R,MR
Picaflor	2021-2420	7	6	86	0-65.5	32.3	0-50	12MR,MS
Dashin	1994-2595	18	17	94.4	0-100	43.3	0-65	13.7MR,MS
Digalu	1961-2006	2	2	100	54-75	64.5	5-25	15MR,MS
Shahan (local)	1912-2654	61	55	90.2	0-100	72.9	0-95	27 S
Ude *	2000-2626	3	3	100	0-10	5	0-10	3.8R
Dembi*	1973-2614	2	2	100	0-5	2.5	0-5	2.5R

and 0-10%, respectively.The lowest incidence and severity of the disease was recorded in Mekelle-4 with mean values of 2.5% each, respectively (Table 9). Moreover, these varieties demonstrated moderately resistant to resistant reaction to leaf rust populations in their inspected fields.

High intensity of wheat leaf rust was found on commercial bread wheat varieties of Dashen, Digalu, Picaflor and Local cultivar as compared with the other varieties. The intensity of the disease in these varieties varied between 0-100% in incidence and 12-27% in severity (Table 9). Varieties, Dashen, Digalu and Picaflor demonstrated moderately susceptible to moderately resistant response to leaf rust across locations. However, the local cultivar consistently showed susceptible response to the disease in all the study areas. As a result, the highest mean incidence of 72.9% and severity of 27% were recorded on this cultivar. The disease was prevalent on 90.2% of the fields cultivated with local cultivar. The long period cultivation and increase in susceptibility from time to time by leaf rust population probably makes the local cultivar highly infected. In addition, the wide cultivation of this cultivar in the study area also played a significant role for its susceptibility, as leaf rust is probably more damaging when large areas are sown to single, genetically homogeneous or closely related cultivars (Ahmad *et al*., 2010). This idea is in line with the reports of Mamluk *et al*. (2000) who stated that, majority of the Ethiopian farmers grow landrace cultivars that are susceptible to the disease; even though a large number of improved cultivars of wheat have been released.

In Ethiopia, leaf rust is one of the most important diseases of wheat and its recurrent outbreaks have threatened wheat production in the country (Bedabo, 2002). For instance, out of the 26 wheat cultivars released in the period 1970 to 1993, only three retained their resistance to leaf rust (Geleta and Tanner, 1995). Generally, most of the varieties demonstrated different response across localities, among varieties and even within the same variety. This variation might be the result of differences in host growth stage. Susceptibility and resistance are often highly correlated with host growth stage even with races specific resistance. A host may be subjected to a heavy inoculum density with favorable infection period at critical growth stage, while other host may not be confronted with similar circumstances when it is at the critical stage (Roelfs, 1992). Environmental difference across districts and variation in aggressiveness among population of wheat leaf rust can also result different responses even with the same varieties.

Identification of P. triticina races in southeastern zone of Tigray

Race analysis provides essential information in determining the range of pathogenic variation in a specific region, screening resistance in cultivars, confirming that host responses are due to race changes, understanding the mechanism of variation as well as in determining the direction of research and breeding programs before the pathogen becomes a threat to wheat crop (Abebe et al., 2013). Using the international system of nomenclature for *P.triticina* (Long and Kolmer, 1989), 22 races were identified from 40 mono pustules or isolates based on their reactions on 16 differential hosts.

Distribution and diversity of P. triticina races across districts

As far as race distribution was concerned, though most of the races were confined to specific districts, some had wider spatial distributions. Four races (FHRT, PHRT, PHTT and THTT) were predominant, representing 55% of the isolates analyzed. Races PHTT and PHRT were the most predominant with frequencies of 20 and 15% respectively, followed by THTT and FHRT with a frequency of 10% each. These races were isolated from three or four districts of the study area (Table 10), which indicated that they were widespread throughout southeastern zone of Tigray. PHTT was detected eight times in the population of wheat leaf rust collected from Wukro, H/wejirat and Enderta districts while, PHRT was isolated six times from S/samre, D/ Temben, Wukro and Enderta populations of wheat leaf rust. On top of this, PHRT was identified as the most distributed race and adapted to wide agro ecologies of the study area. Races, THTT and FHRT also isolated four times each from districts of Wukro, S/samre and H/wejirat and Wukro, Enderta and H/wejirat respectively.

The predominance of races of *P. triticina* in these districts provides evidence of clonal lineages and short distance migration of this pathogen within the study area. On the other hand, approximately 82% of the races including the most virulent race TKTT, were confined to specific locations and detected only once

with a frequency of 2.5% each (Table 10). The distribution and diversity of *P. triticina* races indicated that, genetic similarity among isolates of within and between districts of the study area was existed. The three adjacent districts (Wukro, H/wejirat and Enderta) had two similar races, FHRT and PHTT out of eight, seven and seven races detected, in that order. Likewise, Wukro and S/samre districts had two races in common, PHRT and THTT out of eight each respectively. This genetic similarity between *P. triticina* isolates of these districts is in line with the findings of McVey *et al*. (2004), who reported similar level genetic similarity between *P. triticina* populations collected from Egypt in 1998 to 2000 and from southern and central plains of United States in the same period. The within districts comparison also indicated that, some genetic similarities among isolates of Wukro, H/wejirat, S/samre and D/Temben were observed. Out of the 11 isolates collected in wukro district, 36.4% of the isolates showed genetic similarity and resulted in race PHTT. In H/wejirat district, 20 and 30% of the isolates were resulted in FHRT and PHTT races respectively. This indicated that, only 50% of the leaf rust isolates showed genetic diversity in this district. Isolates of S/samre also showed genetic similarity as a result; races THTT and PHRT were detected two times each from 10 isolates of leaf rust. In the same way, PHRT was identified from two isolates collected from D/ Temben district.

Their geographic proximity, absence of barriers and cultivation of similar bread wheat cultivars among Wukro, H/wejirat and S/samre districts might have played significant role for race similarity. On top of this, these races might be more fit or easily adapted with the environment of these districts. D/Temben district on the other hand is geographically isolated by mountains from other places. Thus, the possibility of migration of urediospores of wheat leaf rust to and from this district is much restricted and low diversity among *P. triticina* population is expected in this district. In contrast, the 'within district' comparison had also indicated that, isolates collected from Enderta showed genetic diversity among the populations of wheat leaf rust. The seven isolates collected from this district yielded seven races (RCJT, PHTT, CBBT, FHRT, MBBR, MGJT and PHRT) (Table 10). The high level of race diversity in this district might be resulted from the windy nature of this area. This area was identified as the second windiest place in Ethiopia. Hence, the movement of *P.triticina* urediospores via wind from their sources to or from this area is a common phenomenon in rusts in general and leaf rust in particular. This circumstance might be resulted, more heterogeneity in the population of wheat leaf rust and finally the chance of detecting different races in this district become increased.

Table 10: Distribution of *P. triticina* races across districts of Southeastern Zone of Tigray

Races	Districts					Isolates	Frequency (%)
	Wukro	Enderta	S/samre	D/Temben	H/wejirat		
BBBT	-	-	1	-	-	1	2.5
BBQR	-	-	1	-	-	1	2.5
CBBT	-	1	-	-	-	1	2.5
FGRT	-	-	1	-	-	1	2.5
FGTT	-	-	1	-	-	1	2.5
FHRT	1	1	-	-	2	4	10
FHTT	1	-	-	-	-	1	2.5
LBBM	-	-	-	-	1	1	2.5
LBDC	-	-	1	-	-	1	2.5
MBBR	-	1	-	-	-	1	2.5
MCST	-	-	-	-	1	1	2.5
MGJT	-	1	-	-	-	1	2.5
MHTT	-	-	-	-	1	1	2.5
PCRR	-	-	-	-	1	1	2.5
PGRT	1	-	-	-	-	1	2.5
PHRT	1	1	2	2	-	6	15
PHTT	4	1	-	-	3	8	20
PJTT	-	-	1	-	-	1	2.5
RCJT	-	1	-	-	-	1	2.5
RHTT	1	-	-	-	-	1	2.5
THTT	1	-	2	-	1	4	10
TKTT	1	-	-	-	-	1	2.5
Total	11	7	10	2	10	40	100

Virulence spectrum of P. triticina races

Virulence spectrum was determined by the number of differential lines that the isolate showed virulence. In this case, an isolate having virulence on more leaf rust resistance genes was considered to have wider spectrum compared to those isolates with virulence to relatively lower number of differential lines (Sewalem *et al*., 2008). In view of this, approximately 73% of the races had virulence spectra raging from 9 to 15 *Lr* genes. The widest virulence spectrum was recorded from TKTT race making 15 *Lr* genes ineffective (Table 11). Though, this race was not widely distributed, it seems to be important in that it attacks all the members of the differential hosts except *Lr9*. In addition, this race has a potential to cause heavy infection on many bread wheat varieties grown in areas where this race was discovered. Similarly, races THTT was also the second most virulent race making 14 *Lr* genes susceptible. The virulence spectrum of *P. triticina* indicated that, some races showed the same virulence spectrum on the *Lr* genes. For instance, three races (RHTT, PHTT and PJTT), (FHTT, MHTT and PHRT) and (FGTT, FHRT and PGRT) were virulent equally to 13 (81.3%), 12 (75%) and 11 (68.8%) of *Lr* genes respectively. Likewise, races FGRT, MCST, PCRR and RCJT had the same virulent spectrum, each produced virulence on 10 or 62.5% of *Lr* genes. Race MGJT was virulent on 9 or 56.3% of the *Lr* genes tested. This indicated that, unless wheat varieties have combined *Lr* genes through pyramiding, the mentioned races above have a potential to cause heavy infection during what production in the region in general and Southeastern zone in particular. In contrast, the remaining six races (BBBT, BBQR, CBBT, LBBM, LBDC and MBBR) or 27% of the races had narrow virulence spectra ranging from 3 to 5 *Lr* genes (Table 11). The “L” group races, LBBM and LBDC were the least virulent, producing compatible reaction only on three *Lr* genes (*Lr1, LrB* and *Lr18*) and (*Lr1, Lr17* and *Lr18*), respectively. Races BBBT, BBQR, CBBT and MBBR were also the least virulent, producing susceptible reactions on four, five, five and five leaf rust resistant genes in that order (Table 11).

Approximately 55% of the races identified in Southeastern zone of Tigray varied from one another by single gene changes. For instance, races FGTT and FHTT were similar to FGRT and FHRT with additional virulence each to *Lr17,* respectively. In the same way, races PHRT, PHTT, THTT and TKTT were similar to PGRT, PHRT, RHTT and THTT with additional virulence to *Lr26, Lr17, Lr2c* and *Lr24,* respectively (Table 11). This slight difference in virulence between these races of leaf rust may resulted from the continuous evolution of leaf rust through one or more of the mechanisms of variation (mutation, migration, recombination and selection pressure on race specific resistance). This idea is in line with the report of Green (1975) who stated that, single step changes in virulence were resulted from the main process of evolutionary change in wheat leaf rust Populations.

The present study indicated that, the identified races of *P. triticina* did not show similarities with the previously identified races in Ethiopia. This could be due to variation over location and time, as races are prevalent in specific season and region depend on the type of wheat cultivars grown (Singh, 1991), and to some extent on the predominant environmental conditions, especially temperature (Roelfs *et al*., 1982). Similar report was also provided by Mengistu and Yeshi (1992) they stated that, a comparison between the races identified in the present study with the earlier reports revealed differences. Generally, the virulence spectrum of the pathogen in this study area confirmed the presence of wider range of virulence among the population of wheat leaf rust races. This might be linked with the fact that, the large population size of leaf rust leads to greater probability of mutants and more diversity of virulence/ avirulence combination existed in the crop (Schafer and Roelfs , 1985).

Virulence frequency of *P. triticina* isolates to *Lr* genes

The result on virulence frequency of *P. triticina* indicated that, majority of the resistance genes were found ineffective by most of the isolates tested in this study. Approximately, 81% of the *Lr* genes were ineffective to more than 55% of the isolates. High virulence (≥72.5%) has been exhibited on *Lr* genes *Lr1, Lr2c, Lr3, Lr16, Lr3ka, Lr11, Lr30, LrB, Lr10, Lr26,* and *Lr14a.* There was 100% frequency of virulence for leaf rust resistant genes *Lr18*. The *Lr*17 has an intermediate virulence frequency of 55%, while the remaining genes, *Lr9, Lr24* and *Lr2a* were found to have between 0-17.5% of virulence frequencies (Table 12). Some *Lr* genes such as, *Lr2c* and *Lr26, Lr16* and *Lr30* and *Lr14a* and *Lr11* had the same virulence frequency of 72.5%, 77.5% and 87.5%, respectively.

Table 11. Virulence /avirulence spectrum of *P. triticina* races collected from Southeastern Zone of Tigray in 2013

No	Races	Virulence (Ineffective *Lr* genes)	AVirulence (effective *Lr* genes)	Virulence factor
1	BBBT	LrB, 10, 14a, 18	Lr1, 2a, 2c, 3, 9, 16, 24, 26, 3ka, 11, 17, 30	4
2	BBQR	Lr3ka, 11, B, 10, 18	Lr1, 2a, 2c, 3, 9, 16, 24, 26, 17, 30, 14a	5
3	CBBT	Lr3, B, 10, 14a, 18	Lr1, 2a, 2c, 9, 16, 24, 26, 3ka, 11, 17, 30	5
4	FGRT	Lr 2c, 3, 16, 3ka, 11, 30, B, 10, 14a, 18	Lr1, 2a, 9, 24, 26, 17,	10
5	FGTT	Lr2c, 3,16, 3ka, 11, 17, 30, B, 10, 14a, 18	Lr1, 2a, 9, 24, 26	11
6	FHRT	Lr2c, 3, 16, 26, 3ka, 11, 30, B, 10, 14a, 18	Lr1, 2a, 9, 24, 17	11
7	FHTT	Lr2c, 3, 16, 26, 3ka, 11, 17, 30, B, 10, 14a, 18	Lr1, 2a, 9, 24	12
8	LBBM	Lr1,B,18	Lr2a , 2c, 3, 9, 16, 24, 26, 3ka, 11,17, 30, 10, 14a	3
9	LBDC	Lr1,17,18	Lr2a, 2c, 3, 9, 16, 24, 26, 3ka, 11, 30, B, 10, 14a	3
10	MBBR	Lr1, 3, B,10,18	Lr2a, 2c, 9, 16, 24, 26, 3ka, 11, 17, 30, 14a	5
11	MCST	Lr1, 3, 26,3ka,11,17, B,10,14a,18	Lr2a, 2c, 9, 16, 24, 30	10
12	MGJT	Lr1, 3, 16, 11, 17, B,10,14a,18	Lr2a, 2c, 9, 24, 26, 3ka, 30	9
13	MHTT	Lr1, 3, 16, 26, 3ka, 11, 17, 30, B, 10, 14a, 18	Lr2a, 2c, 9, 24	12
14	PCRR	Lr1, 2c,3, 26,3ka,11,30,B,10,18	Lr2a, 9, 16, 24, 17,14a	10
15	PGRT	Lr1, 2c, 3,16, 3ka, 11, 30, B, 10, 14a, 18	Lr2a, 9, 24, 26, 17	11
16	PHRT	Lr1, 2c, 3, 16, 26, 3ka, 11, 30, B, 10, 14a, 18	Lr2a, 9, 24, 17	12
17	PHTT	Lr1, 2c, 3, 16, 26, 3ka, 11, 17, 30, B, 10, 14a, 18	Lr2a, 9, 24	13
18	PJTT	Lr1, 2c, 3, 16, 24, 3ka, 11, 17, 30, B, 10, 14a, 18	Lr2a, 9, 26	13
19	RCJT	Lr1, 2a, 3, 26, 11,17, B, 10, 14a, 18	Lr2c, 9, 16, 24, 3ka, 30	10
20	RHTT	Lr1, 2a, 3, 16, 26, 3ka, 11, 17, 30, B, 10, 14a, 18	Lr2c, 9, 24	13
21	THTT	Lr1,2a, 2c,3,16,26,3ka,11,17,30,B,10,14a,18	Lr9, 24	14
22	TKTT	Lr1,2a, 2c,3,16,24,26,3ka,11,17,30,B,10,14a,18	Lr9	15

Table 12: Virulence frequency of *P. triticina* isolates on 16 *Lr* genes in 2013 cropping season

Lr gene	Number of Virulent isolates	Virulence frequency (%)	*Lr* gene	Number of Virulent isolates	Virulence frequency (%)
Lr1	30	75	Lr3ka	33	82.5
Lr2a	7	17.5	Lr11	35	87.5
Lr2c	29	72.5	Lr17	22	55
Lr3	36	90	Lr30	31	77.5
Lr9	0	0	Lr B	39	97.5
Lr16	31	77.5	Lr10	38	95
Lr24	2	5	Lr14a	35	87.5
Lr26	29	72.5	Lr18	40	100

The *Lr18* displayed consistently high infection type to all isolates of *P. triticina* collected from southeastern zone of Tigray. All the identified races including the least virulent races, LBBM and LBDC were virulent on this gene and showed susceptible reaction just like the universally susceptible variety "Morocco". This showed that absolute compatibility of *Lr18* to all races of *P. triticina* was demonstrated. This information may provide a clue either *Lr18* is effective and expressed at adult plant or completely lost from its differential line, RL6009. However, further study on the effectiveness of *Lr18* against wheat leaf rust population is required to consolidate this conclusion. Different authors have reported similar results on the ineffectiveness of the *Lr18*. For instance, Torabi *et al*. (2001) reported that, the host with *Lr18* appeared to be ineffective to all isolates at seedling in the green house, but it showed considerable resistance at adult plant. Singh *et al*. (1991) also reported that virulence status in the pathogen for this gene could not be determined. Similarly, there was also 97.5% frequency of virulence for *LrB.* This gene was found to be effective only to the least virulent race, LBDC isolated from the local cultivar in S/samre district. McIntosh *et al*. (1995) also reported that, *LrB* was ineffective to leaf rust isolates in most geographic areas of Australia. The ineffectiveness of the genes *Lr11* and *Lr17* at seedling stage were expected as they were reported to be adult plant resistant genes (Mesterhazy *et al*., 2000; Kolmer, 2003). Moreover, the ineffectiveness of *Lr1, Lr2c, Lr3* and *Lr10* might be due to these genes have been used in wheat cultivation for many years (Long *et al.,* 1986), during which virulence to these genes become common and most races identified in recent years are virulent to these genes.

Likewise, virulence for *Lr26, Lr16, Lr30, Lr3ka* and *Lr14a* was very common by most isolates of leaf rust with virulence frequencies of 72.5, 77.5, 77.5, 82.5 and 87.5% respectively. This virulence could be result from the fact that, leaf rust differential lines have single and specific resistant genes, and race specific resistant genes have been proven to be very vulnerable to selection and increase virulent races in rust population (Kilpatrick, 1975). On the other hand, *Lr9, Lr24,* and *Lr2a* were found to be effective to most of wheat leaf rust populations (Table12). The leaf rust resistant gene, *Lr9* derived from *Aegiolops umbellulata*, demonstrated an incompatible host-pathogen interaction to all isolates of leaf rust. This implied that, no virulence was observed on *Lr9* (virulence frequency=0%) in all the districts of collection. In Ethiopia, this gene was also found effective to wheat leaf rust isolates collected in 2004 from Ethiopia and Germany (Sewalem *et al*., 2008). This finding is also in a good agreement with the previous studies which stated that, no virulence to *Lr9* was found (Hussain *et al*., 1980). Similarly, *Lr24* was found to confer resistance to 95% of the tested leaf rust isolates. This gene was ineffective only by two virulent races, TKTT and PJTT identified from Wukro and S/samre isolates respectively. Mesterhazy *et al.* (2000) and Kolmer (2003) also stated that, *Lr24* is generally effective in many wheat producing areas of the world. Virulence on *Lr*2a was also rare and found to be effective to 82.5% of leaf rust isolates. Anteneh (2011) also reported that, *Lr2a* was exhibited effectiveness to wheat leaf rust races. Hence, these genes can be used as potential sources during wheat breeding programs for resistance to wheat leaf rust.

Response of wheat varieties to leaf rust races at seedling stage in greenhouse

In this study, seedlings of 10 commonly grown wheat varieties and the universally susceptible check, Morocco were screened against the most virulent and dominant races of leaf rust (TKTT, THTT and PHTT) identified in APPRC greenhouse. The reaction of wheat varieties to leaf rust races in the green house revealed that none of the varieties were immune (no sign of infection to the naked eye) while the infection type varied from 1 (small uredia surrounded by necrotic area) to 4 (large uredia without chlorosis) (Table 13).

Table13. Response of wheat cultivars to dominant and virulent races of wheat leaf rust at seedling stage in greenhouse in 2013 growing season

Cultivar	Races		
	TKTT	THTT	PHTT
Mekelle -1	3	3	2+
Mekelle- 2	3	3-	2
Mekelle- 3	3	3	3
Mekelle -4	3	3	3
Picaflor	3	3+	3
Digalu	3-	2+	2
Dashin	3+	3+	3
Ude	1+	2	2-
Dembi	2-	2-	2-
Local cultivar (Shahan)	4+	4	4
Morocco(Susceptible check)	4+	4	4

"+"=slightly larger than the normal uredinia;
"-"= slightly smaller than the normal uredinia

Among the tested wheat varieties, eight (Mekelle-1, Mekelle-2, Mekell-3, Mekelle-4, Picaflor, Digalu, Dashen and local cultivar), seven (Mekelle-1, Mekelle-2, Mekelle-3, Mekelle-4, Picaflor, Dashen and local cultivar) and five (Mekelle-3, Mekelle-4, Picaflor, Dashen and local cultivar) of them produced susceptible reaction to TKTT, THTT and PHTT races, respectively. Five bread wheat varieties namely, Mekelle-3, Mekelle-4, Picaflor, Dashin and local cultivar were susceptible to the three races. Varieties, Mekelle-1 and Mekelle-2 were susceptible to TKTT and THTT races, but resistant to PHTT. Digalu was only susceptible to TKTT but showed resistance to THTT and PHTT races. The data showed that, the more the virulent race, the more susceptibility on many bread wheat varieties was recorded and vice versa.

Most varieties showed more resistance under the natural infection in the field than at seedling stage in the green house. All the Mekelle varieties, Mekelle-1, Mekelle-2, Mekelle-3 and Mekell-4 showed moderately resistance to resistance under the natural infection in the field, while they showed susceptibility to at least two of the virulent races of leaf rust at seedling stage in the greenhouse. Similarly, Picaflor, Dashin and Digalu showed moderately susceptible to moderately resistance reaction under the natural infection, while they showed susceptible reaction to at least one of the races of leaf rust at their seedling stage.

This variation might be resulted due to, these varieties may have genes responsible for adult plant resistance at field condition, but poorly expressed at their seedlings in the greenhouse. The presence of conducive environment in the greenhouse provided optimum development to leaf rust as compared to the field where environmental conditions might not regulated based on the requirement of the pathogen. Hence, this circumstance also contributed for the susceptible response of these varieties at their seedling stage in the green house. Moreover, varieties in the greenhouse were evaluated through the inoculation of the most virulent and dominant races of leaf rust and resulted susceptible reaction on the seedlings of the above mentioned varieties. However, varieties in the field have a chance to be infected with weak population of leaf rust. In effect, low infection type or resistance to leaf rust could be observed in these varieties under field condition. The local cultivar however, showed susceptible reaction under natural infection and greenhouse inoculations for leaf rust population. This cultivar had high infection types similar to that of "morocco". Therefore, this cultivar can be assumed as having no effective gene (s) at its seedling and adult plant growth stages. Both durum wheat varieties, Ude and Dembi have demonstrated resistance to leaf rust at adult plant in the field and seedling stages in the greenhouse.

In general, durum wheat varieties showed better resistance than bread wheat. This might be associated with the fact that, most of the durum wheat genotypes were developed from local landraces as Ethiopia is the centre of genetic diversity of this species. In effect, indigenous pathogens with high complimentary genetic diversity might co-exist with a wider range of durum wheat genotypes (Tesemma and Bechere, 1998). This idea is also in agreement with previous reports which stated that, most of the commercial durum wheat cultivars exhibited stable resistance to wheat rusts across seasons in hot spot areas of Ethiopia and they could be exploited in wheat breeding programs (Efrem *et al.,* 1995). In contrast, as bread wheat is not indigenous to Ethiopia, cultivars are developed through selection and crossing programs using genetic materials introduced from abroad, mainly from CIMMYT. As a result, bread wheat cultivars in Ethiopia have a narrow genetic base (Hailu, 1991). The narrow genetic base makes bread wheat varieties highly selected and break their resistance by the new race (s) after short period of releasing.

CONCLUSIONS

Leaf rust is one of the most important foliar diseases of wheat in Tigray region in general and south eastern zone in particular. This study indicated that, all the identified races of *P. triticina* have shown genetic uniqueness as compared to the previously identified races in Ethiopia. The variation over location, wheat varieties grown and environmental conditions, especially temperature might be contributed for the uniqueness of the races. Approximately, 81% of the *Lr* genes were ineffective to more than 55% of *P. triticina* isolates. High virulence frequencies (≥72.5%) have been found on the resistance genes *Lr2c, Lr26, Lr1, Lr3, Lr16, Lr3ka, Lr11, Lr30, LrB, Lr10, Lr14a* and *Lr18*. However, *Lr* genes *9, 24* and *2a* were found effective to 100%, 95% and 82.5% of the tested isolates. Hence, these genes are among the most important genes

which could be used as sources of resistance to wheat leaf rust. Evaluation for wheat varieties for their resistances is very important in integrated leaf rust management. In this study, a total of 10 commonly grown wheat varieties were evaluated against three virulent and dominant races of leaf rust at seedling stage in greenhouse and these varieties showed broad infection types from 1 (small uredia surrounded by necrotic area) to 4 (large uredia without chlorosis). Generally, the study confirmed the presence of wider range of virulence among the population of wheat leaf rust races, indicating the presence of genetic diversity among the races in the study area. To conclude, all the tested bread wheat varieties do not have adequate resistances for leaf rust populations, indicating the need for incorporating more effective genes into the target wheat cultivars. However, durum wheat varieties, Ude and Dembi showed resistance to leaf rust population in the field and greenhouse. Hence, they could be important sources of leaf rust resistant genes for this area. Leaf rust is highly variable even within a single cropping season, and breakdown the previously resistant varieties. Hence, it has to be surveyed regularly to determine its current status and to take action before the pathogen becomes a risk to wheat production. Virulence has been observed on all of the *Lr* genes except *Lr9*. Thus, searching for new source of leaf rust resistant genes is necessary to maintain leaf rust resistance. The leaf rust resistant gene *Lr9* was identified as effective gene to all leaf rust isolates. Hence, it should be utilized in breeding program with other effective genes through gene pyramiding as the additive effects of these genes offer the cultivar a wider base of leaf rust resistance. The results from both seedling test and field survey revealed that local cultivar exhibited susceptibility to leaf rust populations. Hence, Breeders and/ Plant pathologists should replace this cultivar by developing resistant varieties that does not follow gene-for-gene specificity. The Ethiopian durum wheat landraces are potential sources of leaf rust resistance. Hence, their resistant genes should be exploited in wheat breeding programs. Finally, plant pathologists and /or breeders should use this data as a base line during resistant variety development program in the study area.

REFERENCES

Abebe T, Woldeab G and Dawit W (2013) Physiological races and virulence diversity of *P. graminis* pers. f. sp. *tritici* eriks. & E. Henn. On wheat in Tigray region, Ethiopia. ESci J. Plant Pathol. 2(1): 01-07.

Afzal SN, Haque I, Ahmedani MS, Munir M, Firdous SS, Rauf, A, Ahmad I, Rattu AR and Fayyaz M (2009) Resistance potential of wheat germplasm (*Triticum aestivum* L.) against stripe rust disease under rain fed climate of Pakistan. Pak. J. Bot. 41(3): 1463-1475.

Ahmad S, Afzal M, Noorka IR, Iqbal Z, Akhtar N, Iftkhar Y and M Kamran (2010) Prediction of yield losses in wheat (*Triticum aestivum* L.) caused by yellow rust in relation to Epidemiological factors in Faisalabad. Pak. J. Bot. 42(1): 401-407.

Anonymous (2007) The State of Food and Agriculture-2007. Food and Agriculture Organization of the United Nations. Rome. pp. 251-252.

Anteneh B (2011) Evaluation of detached leaf assay for assessing leaf rust (*Puccinia triticina* Ericks and Henn.) resistance in wheat. M.Sc. Thesis, School of Graduate Studies of Ambo University, Ethiopia.

Aquino P, Carrion F and Calvo R (2002) Selected wheat statistics. In ''CIMMYT 2000 2001 World Wheat Overview and Outlook: Developing No-Till Packages for Small Scale Farmers''

Ayele B, Eshetu B, Berhanu B, Bekelle H, Melaku D, Asnakech T, Amare A, Kiros M and Fekedu A (2008) Review on two decades of research on diseases of small cereal crops. In Abraham Tadesse (eds.) increasing crop production through improved plant protector Volume I. Proceedings of (4th Annual conference of the Plant protection Society of Ethiopia) 19-22 Dec.2006 Addis Ababa, Ethiopia.PP.598.

Bedabo A (2002) Breeding bread wheat with multiple disease resistance and high yield for the Ethiopian highlands: Broadening the genetic bases of yellow rust and tan spot resistance. Cuvillier Verge, Goettingen, Germany.

Bekele H, Verkuiji H, Mwangi W and Tanner D (2000) Adoption of improved wheat technologies in Adaba and Dodola Weredas of Bale highlands, Ethiopia. Mexico, D.F International Maize and wheat Improvement Center (CIMMYT) and Ethiopian Agricultural Research Organization (EARO).

Belayneh A (2010) Genetic and virulence diversity of *Puccinia graminis f.sp. tritici* population in Ethiopia and stem rust resistant genes in wheat. PhD Thesis. University of Giessen ,Cuviller Verlage Gottingen, Germany. Pp. 1-50.

BoARD (Bureau of Agriculture and Rural Development). (2005) Progress Report on area and productivity of crops in the region.

BFED (Bureau of Finance and Economic Development). (2007) Atlas of Tigray regional state. Ethiopian National Meteorology Agency. Rain fall distribution in Tigray: 1997-2007.

CIMMYT (International Center for Maize and Wheat Improvement). 2005. Sounding the Alarm on Global Stem Rust. An assessment of race Ug99 in Kenya and

Ethiopia and the potential for impact in neighboring regions and beyond. Prepared by the expert panel on the stem rust outbreak in Eastern Africa. 29 May 2005. CIMMYT, Mexico.

CSA (Central Statistical Authority) (2009) Country level Agricultural products producer price index, (Ag-PPI). Addis Ababa, Ethiopia. Pp 20-25.

CSA (Central Statistical Authority) (2011) Report on area and crop production forecast for major crops. Statistical bulletin. Addis Ababa, Ethiopia.

Curtis BC, Rajaram S and Macpherson HG (2002) Bread wheat improvement and production No. 30. Food and Agricultural Organization of the United Nations, Rome Italy pp. 554.

Dagnatchew Y (1967) Plant disease of economic importance in Ethiopia. Exp. Station Bull. No.50.Colege of Agri. Debre Zeit, Ethiopia: H.S.I.U.

Dyck PL and Johnson R (1983) Temperature sensitivity of genes for resistance in wheat to *Puccinia recondita.* Can. J. Plant Pathol.5: 229-34.

Efrem B, Tesfaye T, Mengistu H and Yeshi A (1995) Breeding durum wheat for resistance to leaf rust and stem rusts in Ethiopia. In: Danial DL(ed.). Breeding for disease resistance with emphasis on durability. Proceedings of a regional workshop for Eastern, Central and Southern Africa. October 2-6, Njoro, Kenya. CIMMYT. pp. 125-132.

ENMA (Ethiopian National Meteorology Agency). 2013. Atlas of Tigray regional state. Meteorological data in Tigray: 1997-2013.

Fetch TG and Dunsmore KM (2004) Physiological specialization of *P. graminis* on wheat, barley, and oat in Canada in 2001. Can. J. Plant Pathology. 26: 148-55.

Feyissa R, Kudryavtsev A, Chiapparino E and Chiari T (2005) On-farm conservation and enhancement of local durum wheat genetic resources in Ethiopia. Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress Potenza, Italy, September, 12-15, 2005.

Geleta B and Tanner G (1995) Status of cereal production and pathology research in Ethiopia. P.42-50. In D.L. Daniel (ed.). Breeding for diseases resistance with emphasis on durability. proc. Regional wheat work shop for eastern, Central and Southern Africa, Njoro, Kenya.2-6 Oct.1994. Landbouwuniv. plant breeding Dep., Wageningen, The Netherlands.

Gooding MJ and Davies WP (1997) Wheat production and utilization systems, quality and the environment. Cambridge University Press, UK.

Green GJ (1975) Virulence changes in *Puccinia graminis f.sp. tritici* in Canada. Canadian Journal of Botany 53:1377-1386.

Hailu G (1991) Bread wheat breeding and genetic research in Ethiopia. *In* Hailu, G., Tanner, D.G. and Mangistu, H. (eds). Wheat production and research in Ethiopia: A historical perspectives. Addis Ababa. IAR / CIMMYT. pp. 73-93.

Hussain S, Hassan F and Kirmani MAS (1980) Virulence in *Puccinia recondita* Rob.ex. January 1975 to December 1975. Ambo, scientific phytopathological laboratory.

IFPRI (International Food Policy Research Institute) (2006) Atlas of the Ethiopian Rural Economy. IFPRI and the central statistical agency, Ethiopia. Washington DC. ISBN- 10: 0-89629-154-5.

Kilpatric RA (1975) New wheat cultivars and longevity of rust resistances, 1971-1975. Dep. Agric. Res. Serv. U.S.

Knott DR (1989) The wheat rusts breeding for resistance. Springer- Verlag, Berlin Heidelberg-New York-London-Paris-Tokyo.

Kolmer JA (2003) Postulation of leaf rust resistance genes in selected soft red winter wheat. Crop Sci.43:1266-1274.

Kolmer JA (2005) Tracking wheat rust on a continental scale. Curr. Opin. Plant Biol. 8:441–449. doi: 10.1016/j.pbi.2005.05.001.

Kuraparthy V, Chhuneja P, Dhaliwal HS, Kaur S, Bowden RL and Gill BS (2007) Characterization and mapping of cryptic alien introgression from *Aegilops geniculata* with new leaf rust and stripe rust resistance genes Lr57 and Yr 40 in wheat. Theoretical and Applied Genetics, 114:1379-1389.

Long DL, Schfer JF, Roelfs AP and Roberts JJ (1986) Virulence and epidemiology of *P. recondite f. sp. tritici* in the United States in 1985. Plant dis.70:1107-1110.

Long and Kolmer JA (1989) A North American System of nomenclature for *Puccinia triticina.* Phytopathology, 79*:525-529.*

Madumarov TM (1977) Development of some fungal diseases on cereals in Ethiopia in 1976. EPC Newsletter 4:7-9.

Mamluk OF, El-Daoudi YH, Bekele E, Soth MB, Ahmed MS, Mahir MA and Bahamish HS (2000) Wheat Leaf and Stem Rusts in the Nile Valley and Red Sea Region. ICARDA/NVRSRP, Cairo, Egypt.

Marsalis MA and Goldberg NP (2006) Leaf, stem and stripe rust diseases of wheat. Newsletter Mexico State University Guide A-415.

McIntosh RA, Wellings CR and Park RF (1995) Wheat rusts: An atlas of resistance genes. CSIRO Publications, Kluwer Academic Publishers, Victoria, Australia.

MARC (Mekelle Agricultural Research Center) (1998) Progress Report for 1998. Mekelle, Tigray. Plant Genetic Resources Newsletter 30:33-36.

McVey DV, Nazim M, Leonard KJ and Long DL (2004) Patterns of virulence diversity in *P. triticina* on wheat in Egypt and the United States in 1998-2000: Plant Disease, 88: 271-279.

Mengistu H, Getaneh WA, Rebeka D and Ayele B (1991) Wheat Pathology Research in Ethiopia. *In*: Wheat Research in Ethiopia. A historical perspective. Addis Ababa, IAR /CIMMYT.

Mengistu H and Yeshi A (1992) Variation within indigenous durum wheat germplasm for responses to stem and leaf rust races in Ethiopia. In: Tonner, D.G. and Mwangi, W.(eds.) 7th Regional wheat workshop for Eastern, Central and South Africa. Nakuru, Kenya, CIMMYT Crop development department variety registration, Addis Ababa, Ethiopia.

Mesterhazy A, Bartos P, Goyeau H, Niks RE, Csosza M, Andersen O, Casulli F, Ittu M, Jones E, Menisterski J, Manninger K, Pasquini M, Rubiales D, Schachermayr G, Strzembricka A, Szunics L, Todorova M, Unger O, Vanco B, Vida G and Walther U (2000) European virulence survey for leaf rust in wheat. Agronomy, 20:793-804.

Mideksa F (2011) Assessment and analysis of pathogen virulence and cultivar resistance to wheat leaf rust (*Puccinia triticina* Ericks and Henn.) in Ambo and Toke kutaye districts of Western Showa Zone. Pl. Sc. M.Sc. Thesis presented to the school of Graduate studies of Ambo University, Ethiopia.

Pena RJ (2002) Wheat for bread and other foods. pp 483-494. *In:* Curtis, B. C., Rajaram, S. and Macpherson, H. G. (ed.) Bread wheat improvement and production. FAO, Rome.

Peterson RF, Campbell AB and Hannah AE (1948) A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can. J. Res. Sect. C, 26: 496-500.

Roelfs AP (1992) Effects of barberry eradication on stem rust in the United States. Plant Dis., 66:177-181.

Roelfs AP, Singh RP and Saari EE (1992) Rust diseases of wheat. Concept and Methods of Disease Management. Mexico, DF: CIMMYT.81. pp. 6.

Schafer JF and Roelfs AP (1985) Estimated relation between numbers of urediospores of *P. graminis f.sp. tritici* and rates of occurrences of virulence. Phytopathology, 75:749-750.

Serbessa N (2003) Wheat Stem Rust (*Puccinia graminis* f. sp. *tritici*) intensity and pathogenic variability in Arsi and Bale zones of Ethiopia. Pl. Sc. M. Sc. Thesis presented to School of Graduate Studies of Alemaya University, Ethiopia.

Seck M, Teng PS and Roelfs AP (1985) The role of wheat leaves in grain yield and leaf rust losses. Phytopathology, 75:1299 (abstract.)

Sewalem AM, Heinz WD, Klaus P and Erich CO (2008) Postulation of seedling leaf rust resistance genes in selected Ethiopian and German bread wheat cultivars. Crop Science Society of America, 48: 507-516.

Singh RP (1991) Pathogenecity variation of *Puccinia recondita f.sp.tritici* and *P.graminis f.sp. tritici* in wheat growing areas of Mexico during 1988-1989. Plant disease, 75:790-794.

Singh RP and Rajaram S (1992) Genetics of adult plant resistance in Frontana and three CIMMYT wheat. Genome 35:24– 31.

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA and Ward RW (2008) Will stem rust destroy the world's wheat crop? Advances in Agronomy, 98: 271-309.

Solmatin D and Teman Hussien (1985) The wheat rust situation in Ethiopia during 1984/85. EPC News letter 27: 9-10.

Stakman EC and Levine MN (1962) Analytical key for the identification of physiologic races of *Puccinia graminis tritici*. (Processed) division of cereal crops and dis., USDA, Minnestota Agric. Exp.Sta., pp.7.

Stubbs RW, Prescott JM, Saari EE and Dubin HJ (1986) Cereal Disease Methodology Manual. CIMMYT: Mexico. D.F. pp. 46.

Tesfaye T and Getachew B (1991) Aspects of Ethiopian tetraploid wheat with emphasis on durum wheat genetics and breeding research. *In*: Hailu, G., Tanner, D.G. and Mengistu, H. (eds.) wheat research in Ethiopia: A historical perspective. Addis Ababa.IAR/CIMMYT. Pp. 47-66.

Torabi M, Nazari K and Afshari F (2001) Genetics of pathogenecity of *Puccinia recondita f.sp. tritici,* the causal agent of leaf rust of wheat. Iran. J. Agric. Sci., 32: 635

White JW, Tanner DG and Corbett JD (2001) An agro-climatologically characterization of bread wheat production in Ethiopia. CIMMYT, Mexico NRG-GIS ser.0101.

Winzeler M, Mesterházy A, Park RF, Bartos P, Csosz M, Goyeau H, Ittu M, Jones E, Loschenberger F, Manninger K, Pasquini M, Richter K, Rubiales D, Schachermayer G, Strzembicka A, Trottet M, Unger O, Vida G and Walther U (2000) Resistance of European winter wheat germplasm to leaf rust. Agronomic. 20:783–792. doi: 10.1051/Agro:2000175.

Zadoks JC, Chang TT and Konzak CF (1974) A decimal code for the growth stages of cereals. Plant Dis., 92: 1111-1118.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Nematicidal potential of three plant materials against root-knot nematode (*Meloidogyne incognita*) infestation on tomato (*Solanum lycopersicum*)

Ekpenyong EP, Ononuju CC, Orikara CC and Ikwunagu EA*

College of Crop and Soil Sciences, Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
*Correspnding author Email: emmanuelikwunagu@yahoo.com

Manuscript details:

Received: 15.02.2016
Accepted: 21.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Ekpenyong EP, Ononuju CC, Orikara CC and Ikwunagu EA (2016) Nematicidal potential of three plant materials against root-knot nematode (*Meloidogyne incognita*) infestation on tomato (*Solanum lycopersicum*). *International J. of Life Sciences*, 4(1): 22-28.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

Pot trial experiment was conducted at the College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria between the months of June and October, 2014. The study was designed to determine; the nematicidal effect of leaf powders of *Aspilia africana* (African marigold), *Terminalia catappa* (Indian almond) and *Cymbopogon citratus* (Lemon grass) against nematode infestation on tomato; appropriate time of application of plant materials and to compare the effect of the plant materials with synthetic nematicide (carbofuran). The experiment consisted of six treatments with six replicates, arranged in a Completely Randomized Design. Parameters recorded were number of leaves, plant height, number of fruits, weight of fruits, fresh shoot weight, fresh root weight, number of galls in roots, number of nematodes egg in root and number of nematodes larvae in soil. Result obtained showed that treatments at 1 and 15 days after inoculation (DAI) with nematode eggs significantly reduced nematode population in some parameters than 30 DAI, although in some cases, there were no significant differences between treated and untreated experiments. Thus, different powders significantly increased tomato yield when compared with untreated (nematode alone). However treatments with *Aspilia africana* appeared to be most effective, as it recorded least number of galls irrespective of periods of application, also for eggs in the roots and nematode larvae in soil. Plants treated with nematicide ranked second in reducing number of galls, eggs in roots and nematode larvae in soil. *Aspilia africana* performed better than *Terminalia catappa* and *Cymbopogon citratus* leaf powders, suggesting that they could be used in the management of root-knot nematodes in tomato production as alternative to chemical nematicides in view of their environmental hazard and pollution problems.

Keywords: *Aspilia Africana*, *Cymbopogon citrates*, Carbofuran, *Meloidogyne incognita*, *Terminalia catappa*

INTRODUCTION

Tomato (*Solanum lycopersicum*) is a member of the family *Solanaceae*, as noted by Tindall (2000). It is indigenous to South America, used as food in Mexico and it is grown all over the world following the Spanish colonization of the America (Jones, 2012). The crop has many varieties that are widely grown all over the world, about 100 genera and more than 2,800 species (Griffin and Lin, 2000). They are dicot plants, some have compound leaves while others have simple leaves (Hahn and Fetzer, 2009).

It is the world's largest vegetable crop after Irish potato (*Solanium tuberosum*), but it tops the list of canned vegetables (Fawusi, 1987). In Nigeria, tomato is one of the most important vegetable crop. It is a good condiment in most diets and very cheap source of vitamins A, C and E (Saltveit, 2003). They contain large quantity of water, calcium and Niacin, all of which are very important in the metabolic activities of man. They also contain carotene and lycopene that help in preventing prostate cancer (Mourvaki *et al.,* 2005).

Tomatoes are planted at an estimated rate of 85% each year and produced more in dry season (Allen, 2008). Consumption is in diverse ways, it can be eaten raw, added as ingredient in many dishes, drank as juice, etc. It is cultivated as a major commercial crop in most countries including Nigeria as revealed by Mahovic *et al*., (2004) though it's cultivation is not without limitation, and one of it is disease infestation caused by plant-parasitic nematodes, particularly root-knot nematodes (*Meloidogyne incognita*). This has become the major limiting factor to profitable tomato production (Ononuju, 1999). The nematodes burrow into the soft tissues of roots tips and young roots and cause the nearby root cells to divide and enlarge. Thus infected plants show symptoms like stunted growth, yellowing of the leaves, wilting, and collapse of individual plants swelling or gall on the roots. All root-knot galls damage the vascular tissues of roots and thus interfere with the normal movement of water and nutrient throughout the plants (Olson, 2004). Nematodes generally are regarded as silent enemies, they have caused losses of up to 80% in vegetable fields where their infestation is very high (Kaskavalci, 2007).

Thus goals of controlling nematode is to manage their population, reducing their numbers below damaging levels (Singh and Prasad*,* 2011). They may be prevented by avoiding the introduction of the pathogen into the field, discarding of any transplants showing swelling or galling of the root. Other ways or method whereby nematodes can be controlled include cultural, chemical and biological. Farmers generally have relied on the use of synthetic nematicides over the years and this has resulted to its excessive and unsafe usage (Taniwiryono *et al.,* 2007). Indiscriminate use of synthetic nematicides for the control of nematodes leads to phytotoxicity, environmental pollution and nematodes resistance (Yudelman *et al.,* 1998). On the other hand, its unsafe usage may result in poisoning of humans especially in developing countries like Nigeria (Conway, 1995). To this effect, it is of economic importance to find alternative control strategies which are as effective as synthetic nematicides, safer to farmers, consumers environment and relatively easily available at low price (Fernandez *et al.,* 2001). Such alternatives are the use of biopesticides (nematicides of plant origin) because they are ecological friendly (Javed *et al.,* 2006). Ononuju and Okoye (2003) reported the advantages and potential use of active ingredients of higher plants in controlling plant diseases. Numerous plant species belonging to 57 families have been identified locally to contain nematicidal compounds (Sukul, 1992). They are applied either as soil drench, root dip or as foliar spray (Agbenin, 2004).

The objectives are therefore to;

- determine the nematicidal effects of leaf powders of *Aspilia africana* (African marigold), *Terminalia catappa (*Indian almond*)* and *Cymbopogon citratus* (Lemon grass) on nematode infestation on tomato,
- determine appropriate time of application of the plant powders,
- compare the effect of the plant powders with the synthetic nematicide (Carbofuran).

MATERIALS AND METHODS

Experimental site

The experiment was carried out in the College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Umudike is located on latitude of $5^{0}22^{'}$ North and longitude of $7^{0}33^{'}$ East and an altitude of 122m above sea level. It is situated in the rain forest zone of Southeast of Nigeria,

with an annual rainfall of 1916mm, temperature 19^{0}C to 35^{0}C and a relative humidity of 76% (NRCRI, 2010).

Soil sterilization and packing

Sandy loam soil was steam sterilized and allowed to cool before filling the experimental poly bags with 15kg of soil.

Source of Seeds

Seeds of tomato variety (Admiral) were obtained from the National Horticultural Research Institute (NIHORT) Okigwe Sub-Station Imo State, Nigeria.

Extraction of Nematode Inoculum

Root-knot nematode eggs were extracted from the heavily galled roots of *Celosia argentea* (Soko) using sodium hypochlorite (NaOCl) techniques (Hussey and Barker, 1973). The number of eggs in the suspension on a millilitre was estimated by counting four samples of a millilitre each using down caster counting dish under stereomicroscope and the average was taken. Each millilitre contained 200 nematodes eggs, hence 5mls of the suspension containing 1,000 nematodes eggs were used to inoculate the plants.

Planting of Seeds

The seeds of tomato were planted in the nursery, four weeks after germination, healthy plants were transplanted one per poly bag filled with 15kg sterilized soil.

Inoculation of Nematode Eggs to the Plant

Two weeks after transplanting, the plants were inoculated by pouring a calculated volume of the suspension containing 1,000 nematode eggs extracted by Hussey and Barker (1973) method near the plant by making a groove around it.

Experimental Design

The experiment was arranged in a Completely Randomized Design (CRD) in an open field platform using poly bags with six treatments replicated six times including the control.

Treatment application

The six treatments were as follows:

T1 = Nematode eggs + 30g *Aspilia africana* leaf powder

T2 = Nematode eggs + 30g *Terminalia catappa* leaf powder

T3 = Nematode eggs + 30g *Cymbopogon citratus* leaf powder

T4 = Nematode eggs + 0.3g ai synthetic nematicide (carbofuran)

T5 = Untreated control (Nematode only)

T6 = Uninoculated control (Nematode and treatment free)

The applications of the treatments were done in three phases:

PHASE 1: Treatments were followed immediately after inoculation (1 DAI) of Root-knot nematode eggs.

PHASE II: Treatments were applied 15 days after inoculation (15 DAI) of the Root-knot nematode eggs.

PHASE III: Treatments were applied 30 days after inoculation (30 DAI) of the Root-knot nematode eggs.

Preparation of Plant Extracts

Fresh plant materials were collected and air-dried before they were blended to powder with hand blender. Then 30g of each of the extracts powder were weighed separately and mixed in the sterilized soil in the poly bag at the appropriate time interval.

Data collection

The following data were collected at the end of the experiment: Number of leaves, plant height (cm), number of fruits, weight of fruits (g), fresh shoot weight (g), fresh root weight (g), number of galls in roots, number of nematode egg in roots and number of nematode larvae found in soil.

Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) and means were compared using Least Significant Difference (LSD) at 5% probability level ($P \leq 0.05$) by using Genstat Discovery Edition 4 Statistical Package.

RESULTS

The effect of treatments on plant height, number of leaves and fresh shoot weight applied at different periods after inoculation of plants with eggs *of Melodoidogyne incognita* as shown in Table 1. The treatment did not result in significant differences in all the parameters irrespective of the period of application. However, uninoculated and control experiments recorded the highest plant height (58cm, 61cm, and 63cm), number of leaves (20.00, 18.00, and 19.50) and fresh shoot weight (187.80g, 91.10g and 76.25g) at 1, 15, and 30 DAI respectively, while the least parameter were recorded in plants nematodes alone for plant height and number of leaves.

Table 1: Effect of treatments on plant height, number of leaves and shoot weight after 1, 15 and 30 days after inoculation

Treatment	Plant height (cm)			No of leaves			Fresh shoot weight (g)		
	1 DAI	15 DAI	30 DAI	1 DAI	15 DAI	30 DAI	1 DAI	15 DAI	30 DAI
T_1	30.00	35.00	53.00	16.50	18.00	12.50	117.10	68.60	41.65
T_2	43.50	56.50	40.00	15.00	15.00	11.00	97.95	78.40	39.60
T_3	45.00	42.00	59.00	12.01	13.50	12.00	55.75	78.10	42.00
T_4	52.00	59.00	37.00	16.02	14.50	11.50	33.95	67.20	48.35
T_5	22.00	26.00	27.00	5.00	8.50	8.50	43.20	47.30	52.25
T_6	58.00	61.00	63.00	20.00	18.00	19.50	187.8	91.10	76.25
LSD 0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 2 : Effect of treatments on fresh root weight, number of fruits and weight of matured tomato fruit after 1, 15 and 30 days after inoculation.

Treatment	Fresh root weight (g)			Number of fruits			Weight of fruit (g)		
	1 DAI	15 DAI	30 DAI	1 DAI	15 DAI	30 DAI	1 DAI	15 DAI	30 DAI
T_1	6.15	14.24	14.06	12.50	15.00	13.00	72.25	172.40	137.65
T_2	28.90	22.65	15.30	10.50	7.50	8.30	77.60	116.55	94.8
T_3	12.55	18.00	13.85	17.50	14.50	13.50	177.30	168.60	201.4
T_4	13.10	14.30	13.70	14.00	10.50	9.50	167.10	193.10	120.4
T_5	59.45	45.25	55.95	4.50	3.00	2.50	41.95	45.00	34.00
T_6	29.85	20.10	17.10	16.00	15.00	12.50	153.90	141.70	200.8
LSD 0.05	30.23	20.50	16.24	6.32	4.60	NS	70.20	77.00	68.40

Table 2 shows the effect of treatments on fresh root weight, number of fruits and weight of fruits. There were significant differences among the treatments. Although, highest root weight were recorded in untreated plants (nematode alone, 59.45, 45.25, and 55.95g), while lowest root weight were observed in plant treated with *Aspilia africana* at 1 DAI (6.15g) and 15 DAI (14.24g) respectively, while at 30 DAI, the lowest was seen in plant treated with nematicide (13.70). Similar observations were recorded in weight of fruits, all the treatments were significantly different with the highest weight of fruit coming from plant treated with *Cymbopogon citratus* at 1 DAI (177.30g) and 15 DAI (193.10g). While that of 30 DAI was from uninoculated plant (200.8g). The lowest weights of fruit were noticed in untreated plants (nematode alone), 41.95g, 45.00g and 34.00g). On number of fruits, significant differences were recorded at 1 DAI and 15 DAI of treatments with highest number of fruits recorded from uninoculated plants (16.00 and 15.00), and the lowest recorded in plant with nematode alone (4.00 and 3.00). While at 30 DAI, there was no significant difference, though highest fruit was seen in plants treated with *Cymbopogon citratus* (13.50) and lowest in nematode alone (2.50).

The result of treatments on number of galls in roots, nematode eggs in roots and nematodes larvae in soil are presented in Table 3.3. From the Table, it is shown that number of galls were significantly higher in plant with nematode alone, thus in all the 3 phases (days of treatment application) plant treated with *Aspila africana* showed no gall (0.00) at 15 DAI. It also recorded the least number of galls at 1 DAI (4.00) while at 30 DAI was seen with plants treated with nematicide (5.00). Although there were significant differences shown among all the treatments except at 30 DAI. Eggs in the roots of tomato crop treated with *Aspilia africana* showed great reduction of egg population (150) though significantly different from other treatments, except plant with nematode alone at 15 DAI. Plants treated with *Aspilia africana* recorded zero (0) egg population and was significantly different. However at 30 DAI, plants treated with nematicide (carbofuran) had least number of egg population (350), they were all significantly different. Finally, observations on nematode larvae were significantly reduced in both plant treated with *Aspilia africana* (50.00) and nematicide (50) at 1 DAI. Although *Cymbopogon citratus* and *Terminalia catappa* also reduced nematode population.

Table 3: Effect of treatments on number of galls in root, nematode eggs in roots and nematode larvae found in each tomato pots after 1, 15 and 30 days after inoculation.

Treatment	Number of Galls			Eggs in Roots			Nematode larvae in soil		
	1 DAI	15 DAI	30 DAI	1 DAI	15 DAI	30 DAI	1 DAI	15 DAI	30 DAI
T_1	4.00	0.00	9.50	150	0.00	500	50.00	50.00	50.00
T_2	18.00	40.00	12.50	750	2550	6500	100.00	13.50	50.00
T_3	18.50	24.50	11.50	1000	1250	500	26.00	700	100.00
T_4	14.00	13.50	5.00	1300	950	350	50.00	150	0.00
T_5	132.50	140.00	62.00	8700	5900	2100	1095.0	2600	1250
T_6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD 0.05	40.98	53.68	NS	3,373	3,451	1,653.0	3,735	NS	NS

DISCUSSION

This study has shown that nematode population density applied had damage symptoms on the crop, indicating that the higher the population density of nematode infestation in the field, the higher the extent of damage on crops. This confirms the findings of Ononuju and Fawole, (2000) who reported that the extent of damage by nematode is influenced by the level of soil nematode infestation and environment factors. The results obtained in this study varied among the different days of treatment application. The crop treated with plant extract showed better growth effects than the untreated crops (Chitwood, 2002). Observation from this Study further revealed that in the absence of root- knot nematode infestation and infection, tomato yield will be better since there would be no damage through gall incidence.. This was shown in the poly bag without nematodes which had the best yield.

Aspilia africana treatment improved fruit yield. This means that they reduced nematode population beyond the level at which they would have caused economic damage. This fact was confirmed in the result from the poly bag with nematodes alone which gave the least fruit yield due to nematode population that was high enough to cause economic losses or damage. The superior growth observed in some crops with treatment which is similar to uninoculated crops was due to reduction in a disease effect or destruction of *Meloidogyne incognita* which in turn promoted growth and yield of tomato (Hoseinpoor and Kargar, 2012). Wafaa and Mahmoud, (2013) reported the importance of various soil amendments with the plant botanical and their impact in reducing nematode population and their build up in soil and consequence increase in crop yield.

The study also indicated that there were no significant differences among the treatments on plant height, number of leaves and fresh shoot weight. Observations on these parameters in this study was similar to that of Trudgill and Philips, (1992) who confirmed that nematode infestation led to wilting and stunted growth. There were no significant differences in number of galls, number of fruits and nematode larvae in soil, at 30 DAI, because the tomato plants must have been severely damaged by nematodes infestation (i.e. economic injury level (EIL) before applying the control measures, which led to reduction in resistance by the plant. The root-knot nematode *Meloidogyne incognita* has been reported by many workers, as an important root parasite infecting tomato thus reducing yield upto 28-68% (Pakeerathan *et al.,* 2009). Also, the extent of galling on roots with corresponding heavy weight of roots recorded in control experiments with only nematode inoculum is a means of detecting the infestation of *Moloidogyne* species and the damage caused.

Invariably, infestation of nematode galls, egg population and larvae production were significantly reduced mostly at 1 and 15 DAI, in some cases. This could be that the plants were treated earlier and in addition with the effects of various treatment combinations. The plant powders were highly effective in their ability in reducing root galls, egg mass and population of nematode in soil when compared with the untreated crops (Nematode alone) this suggest powders of the test plants must have been toxic to eggs and or juveniles of nematodes thus reducing the nematode population density as well as galling. Similar results have been reported by other research workers

(Babatola, 1990, Akhatar and Alam 1993, Alem *et al.,* 1994).

The effects of the tested plant extracts varied in their toxicity, *Aspilia africana* was highest in potency, followed by *Cymbopogon ciratus* and the *Terminalia catappa.* This could be due to differences in their chemical composition and concentration of toxic components. Such results have also been reported by Firoza and Magbool (1996) where they used different plant extracts against *Helicotylenchus dihystera* in tomato. *Aspilia africana* was comparable to carbofuran (chemical nematicide) in efficacy which suggest the need for its use as an alternative to synthetic chemicals in tomato production. Hence the need for an increased research on botanicals nematicides for the control of nematodes.

CONCLUSIONS

The present study showed that plant extracts present were cheap and effective nematicides for root knot nematode control. The extracts of *Aspilia africana, Cymbopogon citratus* and *Terminalia catappa* were found to have nematicidal properties. However, the treatment *Aspilia africana* was significantly most effective at the different days of treatment application. *Aspilia africana* reduced nematodes infestation at zero (0) level and increased total tomato yield. This can be used effectively for the management of root-knot nematode in edible tomato without use of chemical nematicide in view of their hazardous effects in the environment. It will also help farmers to shift from the concept of control to the concept of management which is a procedure directed to reducing and maintaining the number of plant-parasitic nematodes at non-injurious level.

However, further rates of application of treatments are needed in both greenhouse and field trials also, the appropriate application time should be further evaluated.

REFERENCES

Agbenin NO (2004) Potentials of organic amendment in the control of plant-parasitic nematodes. Plant protection Sci. 40:21-25.

Akhatar M, Alam MM (1993) Utilization of waste materials on nematode control. A review Biore. Technol 47: 1-17.

Alem MM, Siddiqui SA, Khan MA (1994) Mechanism of control of the plant parasite Nematode as a result of organ amendment to soil, III role of Phenol in host roots. Indian Journal Nematol. 7: 23-31.

Allen A (2008) “A Passion for tomato” smithsonia magazine. Pp. 26-28

Chitwood DJ (2002) Phytochemical based strategies for nematode control. Ann Rev phytopathol. 40: 221-249.

Conway G (1995) The depletion of natural resources: the impact of food. A 2020 vision for food, agriculture and the environment. International food policy research institute, Washington D.C, p 125.

Fawusi MOA (1987) Emergence and seedling growth of pepper as influence by soil compaction nutrient status and moisture regime Soc. Hortic., 9:329-335.

Fernandez C, Rodriguez-Kabana R, Warrior P, Kloeppe JW. (2001) Induced soil suppressiveness to a root-knot nematode species by a nematicide. Biol. Control. 22(2):103-114.

Firoza K, Magbool MA (1996) Effect of Plants Extracts in the control of *Helicotylenchus dihystera* (Cobb, 1893) Sher 1961, Park, Nematol 14: 61-66.

Griffin LUS, Lin GD (2000) Hand Book on tomato production.

Hahn J, Fetzer J (2009) “Slugs in home gardens” university of Minnesota extension. Archived from the original on 2011-03-11 retrieved 23 June 2012.

Hoseinpoor R, Kargar A (2012) Evaluation of the effect powder and aqueous extracts of some plant species on tomato yield and reproduction of *Meloidogyne incognita.* International journal of Agric. Sci. Vol. 2(10): 964-968.

Hussey RS, Barker KR (1973) A comparison of methods of collecting inocula for *Meloidogyne spp.* including a new technique. Plant disease Reporter 57:1025-1028.

Javed N, Gowen SR, Inam-ul-haq M, Abdollah K, Shahina F. (2006) Systemic and persistent effect of neem (*Azadirachta indica*) formulations against root-knot nematodes, *Meloidogyne javanica* and their storage life. Crop protection 26: 911-916.

Jones JB (2012) “Growing in the greenhouse” growing tomatoes. Com. Retrieved 14 August, 2012.

Kaskavalci G (2007) Effect of soil solarization and organic amendment treatments for controlling *Meloidogyne incognita* in tomato cultivar in Western Anatolia. Turkish Agricultural Forum 31: 159-167.

Mahovic MJ, Sargent SA, Bartz JA (2004) Identifying and Controlling Post-Harvest Tomato Disease in Florida. Publish north Carolina Co-operative Extension Service.

Mourvaki E, Gizzi S, Rossi R, Rufini S (2005) “Passion-flower fruit- A New Source of lycopene?” Journal of

Medicinal food 8(1): 104-106. Doi: 10.1089/jmt. 2005. 8 104. PMID 15857218.

NRCRI (2010) National Root Crop Research Institute NRCRI Umudike Metrological Station Data, 2010.

Olson SN (2004) Physiological, Nutritional, and Other Disorders of tomato Fruit. HS-945. Horticultural sciences Department, Florida Co-operative extension services, university of Floride.

Ononuju CC (1999) Management of plant-parasitic nematode problems in commercial plantain and Banana Productions. Paper presented and the 3rd Annual conference of Agric society. Badeggi, Nigeria October 18-22.

Ononuju CC, Okoye CD (2003) Nematoxic activity of some common weed extracts against root-knot nematode in soyabean. *Journal Agricultural and environment;* Afri. Appl. 2001. Environ. Biol. 5: 64-66.

Pakeerathan K, Mikunthan G, Tharshani N (2009) Effect of different animal manures on tomato. World and Agric. Sci. 5(4): 432-435.

Saltveit ME (2003) Mature Fruit vegetable. In post-harvest physiology and pathology vegetable. J.A. Bartz and J.K. Brecht (Eds). Marcel Dekker, New York.

Singh JK, Prasad UK (2011) Crop Diseases and Control Enkay Publishing House, New Delhi India P. 123.

Sukul NC (1992) Plant antagonistic to plant parasitic nematode. Indian Rev. Life Sci. 12: 23-52.

Taniwiryono WD, Brink PVD, Rietjens IMCM, Murk AJ (2007) A case study in Bangka Island, Indonesian on the habits and consequences of pesticides use in black pepper plantations. J. Environ. Toxicd. 22(4): 405-414.

Tindall HD (2000) Vegetable in the tropics. Pp 326-327.

Trudgill DL, Phillips MS (1992) Nematode population dynamics, threshold levels and estimation of crop losses. Food and Agriculture organization of the United Nations, Rome, Italy.

Wafaa MAE, Mahmoud MAY (2013) Comparative efficacy of garlic cloves and easter seed aqueous extracts against the root-knot nematode, *Meloidogyne incognita* infection tomato. Journal of Plant Protection Research 53(3):285-288.

Yudelman M, Ratta A, Nygard D (1998) Pest Management and Food Production: Looking to the future, food, Agriculture and the environment Discussion Paper 25, International Food Policy Research Institute, USA, p. 50.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Assessment of Phytochemicals and Antioxidant activities of *Leucas indica* aerial parts- A comparative study

Sowjanya M[1*], Kiran Kumar M[1] and Sandeep BV[2,]

[1]Department of Biotechnology, College of Science and Technology, Andhra University, Visakhapatnam-530003, India.
[2]Department of Biotechnology, College of Science and Technology, Andhra University, Visakhapatnam-530003, India.
*Corresponding author Email: vijaya.no286@gmail.com)

Manuscript details:

Received: 05.02.2016
Revised: 29.02.2016
Accepted :15.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Sowjanya M, Kiran Kumar M and Sandeep BV (2016) Assessment of Phytochemicals and Antioxidant activities of *Leucas indica* aerial parts- A comparative study. *International J. of Life Sciences*, 4(1): 29-43.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

The exploration was to evaluate the phytochemical ingredients and antioxidant activities of annual herb *Leucas indica* methanolic leaves and flowers extracts. These were estimated by distinguished methods. Phytochemical constituents and non enzymatic antioxidants showed enhancement in increasing concentration from 25 mg/ml to 100 mg/ml. All the assays were carried out in triplicate and the results uttered as mean values ± standard deviations. The levels of phytochemical, explicitly phenolics, flavonoids, tannins, alkaloids, saponins, vitamin C, β-Carotene and lycopene, evidently exposed that methanolic leave extract had elevated phytochemical constituents than methanolic flower extract, except alkaloids. Same pattern was observed for antioxidant capacity assays, specifically DPPH, FRAP, reducing power assay, Hydroxyl radical scavenging activity and ABTS radical scavenging activity, this may be due the presence of high phytochemical ingredients. Even though, leave extract had superior activities than flowers extract, they both have profound therapeutic potential.

Keywords: Antioxidants, ABTS, butylated hydroxy toluene, β-Carotene, phytochemicals, rutin and lycopene.

INTRODUCTION

A complete storehouse of remedies has been provided by nature to cure ailment of mankind (Kumar and Chandrashekar, 2011) one of which includes medicinal plant. Medicinal plants contribute drugs in the form of phytochemcals, which are reported to have various biological activities (Samy *et al.,* 2008). Phytochemcals may minimize the production of reactive oxygen species ROS or it may protect itself from deleterious effect of ROS by efficiently scavenging of ROS. The scavenging system controlling reactive oxygen species (ROS) comprises of antioxidant components (Sharma, 2013). Free radicals act as a trigger to a number of degenerative diseases. Therefore, samples having free radical scavenging activity can be of potent medicinal importance (Chen and Ho, 1995).

Leucas indica (family- Lamiacae) is an annual herb found throughout India as a weed in cultivated fields, wastelands and roadsides. The flowers are given with honey to treat cough and cold in children. The leaves are applied to the bites of serpents, poisonous insects and scorpion sting. *L. indica* leaves are also used as insecticides and mosquito repellent in rural area. The plant extract with honey is a good remedy for stomach pain and indigestion (Madhava Chetty *et al.,* 2008). In the underway effort a comparative cram was conceded out in between, methanolic leave and flower extracts of *Leucas indica* for their phytochemicals and antioxidant activities.

MATERIALS AND METHODS

Plant material:

The Arial parts, leaves and flowers of *Leucas indica* Linn were collected from Marlapudi village, Nellore district, Andhra Pradesh. India, in the month of November – March, 2015. Plant materials were recognized with the help of Gamble, "Flora of the Presidency of Madras" and later verified by comparison with the authentic specimens available in the herbariums of National Botanical Research Institute (NBRI), Lucknow and these plant materials were authenticated by Dr. M. Venkaiah, faculty of Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. Leaves and flowers of *Leucas indica* were cleaned, shade dried, mechanically grinded. Finally, the coarse powders were separated by sieving and stored in an air tight container for further use.

Preparations of extracts:

Accurately weighed 10 gms of *Leucas indica* leave and flower powder were extracted with 250 ml methanol by stirring at 50^{oC} for 3hr. The extracts were then filtered through whatmann filter paper and the filtrate was concentrated with a vacuum rotary evaporator under low pressure and temperature and stored in desiccator.

Evaluation of Phytochemicals

Estimation of total phenolics

The amount of total phenolics in extracts was determined according to the Folin- Ciocalteu procedure (Javanmardi *et al.,* 2003). Samples (200 µl) were introduced into test tubes. One milliliter of Folin Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured. The total phenolic content was expressed as gallic acid equivalents (GAE) in micrograms per gram of extract as calculated from standard gallic acid graph.

Estimation of total flavonoids

Total flavonoid content of the extract was determined according to a modified colorimetric method (Bao *et al.,* 2005). Test extract (1.0 ml) was mixed with 1ml of distilled water and 75 µl of a 5% $NaNO_2$ solution. After 5 min, 75 µl of 10% $AlCl_3.H_2O$ solution was added. After 5 min, 0.5 ml of 1M Sodium hydroxide was added. The solution was mixed well and kept for 15 min. The increase in absorbance was measured at 510 nm using a UV-Visible spectrophotometer. The total flavonoid content was calculated using standard quercetin calibration curve. The results were expressed as micrograms of quercetin equivalents (QE) per gram of extract.

Estimation of total tannins

The total tannins were determined using the Folins-Ciocalteau method (1927), briefly, 0.1 ml of test extract, 6.5 ml of water and 0.5 ml of Folins-Ciocalteau reagent and 1.5 ml of 20% sodium carbonate at overnight standard solution were added and incubated at 1 h the absorbance of sample was measured in spectrophotometer at 725 nm. The total tannin content was calculated using standard tannic acid calibration curve and the results were expressed as micrograms of tannic acid equivalents per gram of extract.

Estimation of total alkaloids

Total Alkaloid content was estimated by the method of Sreevidya and Mehrotra (2003). A standard solution was prepared by dissolving 5 mg of boldine and test extracts separately in 5 ml of warm distilled water each. Five ml of boldine solution/extract was adjusted to PH 2-2.5 (with 0.01 M HCl), and 2 m of DR (Dragendorff's reagent) was added to form an orange precipitate that was centrifuged at 5000 rpm for 15 min. Afterward, DR was added to the supernatant to check for complete precipitation. 2 ml amount of 1% sodium sulfide was added to the residue to form a brownish black precipitate which was centrifuged at 5000 rpm for 15 min. Complete precipitation was checked by further adding 1% sodium sulfide. The resulting residue was dissolved in 2 ml of nitric acid with warming and sonication and then made up to 10

ml with distilled water.5 ml of 3% thiourea was added to 1 ml of the resulting solution to form a yellow bismuth complex, of which the absorbance was measured at 435 nm. The amount of bismuth present in the boldine solution/extract was achieved from the calibration curve of bismuth nitrate. The results were expressed as boldine, considering that is a monobasic alkaloid, and therefore the complex formed with bismuth follows a 1:1 stoichiometry.

Estimation of Saponins

The method used was that of Obadoni and Ochuko (2001). 25 g, 50g and 100g of samples powder were put into conical flasks separately and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

Estimation of Vitamin C:

Ascorbic acid content was determined by the procedure described previously (Sadasivam and Theymoli, 1987). Briefly, to 5.0 ml of ascorbate solution (10µg per ml), 10 ml of 4% oxalic acid was added and titrated against 0.026% dichlorophenol indophenol. The amount of the dye consumed was equivalent to the amount of ascorbic acid present in the plant extracts. Similar titration was carried out with 5.0 ml of leave and flower extracts alone.

Estimation of β-Carotene and lycopene:

β-Carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The dried methanolic extract (100 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/ 100 ml) = 0.0458 A_{663} + 0.372 A_{505} 0.0806 A_{453}; β-carotene (mg/100 ml) = 0.216 A_{663} 0.304 A_{505} + 0.452 A_{453}. Results were expressed as µg /gram of extract.

Antioxidant capacity assays

Diphenyl picryl hydrazyl radical scavenging Assay (DPPH)

3 ml of test extract was added to 1ml of 0.1 mM solution of DPPH in methanol. After 30 min incubation at 37°C absorbance was measured at 517 nm against control using a spectrophotometer (Cuendet *et al.,* 1997). Rutin and BHT were used as the reference materials. The percentage of inhibition was calculated by comparing the absorbance values of the test samples with those of the controls. The inhibition percentage (I) was calculated as radical scavenging activity as follows

Percentage of inhibition (I) = (Absorbance of Control-Absorbance of Test)/ Absorbance of control × 100

Ferric reducing or antioxidant power assay (FRAP)

The total antioxidant power of the sample was assayed by the method of Benzie and Strain (1996). 3.0 ml of FRAP working reagent was taken in a test tube then 100 µl of test extract was added, this is vortex mixed, and the absorbance was read at 593 nm against a reagent blank at a predetermined time after sample-reagent mixture. The results are expressed as Ascorbic acid equivalents (µ moles/ml) or FRAP units.

Determination of Iron (III) to Iron (II) Reducing Activity (or) reducing power assay

The ability of the extracts to reduce iron (III) was assessed by the method of Oyaizu (1986). One ml of seed extract was mixed with 2.5 ml of 0.2 M phosphate buffer, PH 6.6, and 2.5 ml of 1% aqueous potassium hexacyanoferrate [$K_3Fe(CN_6)$] solution. After 30 min of incubation at 50 °C, 2.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged for 10 min. Finally, 2.5 ml of the upper layer was mixed with 2.5 ml of water and 0.5 ml of 0.1% aqueous $FeCl_3$, and the absorbance was recorded at 700 nm using a UV-Visible spectrophotometer. Increasing absorbance at 700 nm was interpreted as increasing reducing activity. The results were expressed as micrograms of ascorbic acid equivalents (AscAE) per gm of extract. Butylated hydroxy toluene (BHT) and ascorbic acid were used as positive controls. Ascorbic acid was used

as the standard control with concentrations 10, 20, 40, 60, 80 and 100 µg/mL.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/Ascorbate/EDTA/H_2O_2 system, a method carried out by Gulhan *et al.,* (2003). 0.1 ml of the test extract was added to the reaction mixture containing 0.1ml of 3.0 mM deoxyribose, 0.5 ml of 0.1 mM $FeCl_3$, 0.5 ml of 0.l mM EDTA, 0.5 ml of 0.1mM ascorbic acid, 0.5 ml of 1mM H_2O_2 and 0.8 ml of 20 mM phosphate buffer, PH 7.4, in a final volume of 3.0 ml. The reaction mixture was incubated at 37 ^{o} C for 1 h. The formed thiobarbituric acid reactive substances (TBARS) were measured by adding 1.0 ml of thiobarbituric acid (TBA) and 1.0 m of trichloroacetic acid (TCA) to the test tubes and incubated at 100 ^{o} C for 20 min. After the mixtures were cooled, absorbance was measured at 532 nm against a control containing deoxyribose and buffer. A blank was carried out similar way as the test except test compound. Inhibition (I) of deoxyribose degradation in percent was calculated in the following way.

I= (Absorbance of control –Absorbance of test / Absorbance of control) x100.

ABTS radical scavenging activity

ABTS radical scavenging measurements were performed according to Re *et al.,* (1999) with modifications described previously (Barton´, Fołta and Zachwieja 2005). ABTS radical cation was generated by the interaction of ABTS and $Na_2S_2O_8$. For measurement of sample scavenging activity, 2 ml of ABTS were added to the cuvettes containing the pre-diluted samples (0.15, 0.3, 0.45, 0.6, 1.0 ml extracts with addition of 0.85, 0.7, 0.55, 0.4, and 0 ml (phosphate buffered saline) PBS respectively. The absorbance was measured after 6 minutes at the wavelength of 734 nm. The total antioxidant capacities (TAC) were estimated as (6-hydroxy-2, 5, 7, 8-tetramethyl-2-carboxylic acid) trolox equivalents (TEAC) interpolation to 50% inhibition (TEAC50).

RESULTS AND DISCUSSION

Evaluation of phytochemicals and Antioxidant components

The lofty phytochemicals and antioxidant components in the *Leucas indica* methanolic leave and flower extracts indicate that these bioactive agents might partly be responsible for the folkloric use of the fractions in traditional medicine. All the above assays were carried out in triplicate and the results expressed as mean values ± standard deviations. The levels of phytochemical constituents of analysis mentioned below were at 100 mg/ml. The total phenolic content of leaves was 4.42 ± 0.03µg of gallic acid equivalents g-1 of extract whereas for flowers, it was 2.37 ± 0.05µg of gallic acid equivalents g-1 of extract. Our findings showed in figure: 1(a).

Table: 1 (a): Total phenolic content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg of gallic acid equivalents/gram of extract	Flowers µg of gallic acid equivalents/gram of extract
25	1.59 ± 0.03	0.68 ± 0.02
50	3.70 ± 0.02	1.83 ± 0.04
100	4.42 ± 0.03	2.37 ± 0.05

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	12.970	2	6.485	8.338E3	.000
	Within Groups	.005	6	.001		
	Total	12.975	8			
Flowers	Between Groups	3.867	2	1.934	1.513E3	.000
	Within Groups	.008	6	.001		
	Total	3.875	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

Table: 1 (b): Total flavonoid content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg of quercetin equivalents/gram of extract	Flowers µg of quercetin equivalents/gram of extract
25	7.48 ± 0.20	7.35 ±0.45
50	15.06 ± 0.35	14.53 ± 0.30
100	22.82 ± 0.32	22.40 ± 0.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	353.142	2	176.571	1.986E5	.000
	Within Groups	.005	6	.001		
	Total	353.148	8			
Flowers	Between Groups	340.140	2	170.070	1.531E5	.000
	Within Groups	.007	6	.001		
	Total	340.147	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

Table: 1 (c): Total tannin content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg tannic acid equivalents/gram of extract	Flowers µg tannic acid equivalents/gram of extract
25	0.56 ±0.03	0.42 ±0.02
50	0.94 ± 0.03	0.84 ± 0.02
100	1.56 ± 0.02	1.15 ± 0.02

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	1.538	2	.769	1.473E3	.000
	Within Groups	.003	6	.001		
	Total	1.541	8			
Flowers	Between Groups	.833	2	.416	1.171E3	.000
	Within Groups	.002	6	.000		
	Total	.835	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

Phenolics or phenolic acids, intermediates in phenylpropanoid metabolism, play many important roles in plant cells, tissue and organs (Dixon and Paiva 1995). Earlier discoveries have revealed that phenolic compounds are involved in plant development during seed germination and in plant-microbe recognition and signal transduction (Lynn and Chang 1990). Phenolic acids and flavonoids are the most persistent group of plant phenolics that play significant role in plants, for human health and function as reducing agents and as free radicle scavengers (Mamta *et al.,* 2012). Flavonoids were remarkable reactive oxygen

species scavengers and fight continuously against polluted atmosphere. These metabolites were effective in temperature stress, drought situation, freezing injuries of cell membranes and unusual salinity. Flavonoids act as signal molecules to take preventive measures in order to save them from pathogenic microbial attacks (Shirley, 1998).Recent studies have reaffirmed the link between flavonoids and plant architecture by showing that flavonoid-defective mutants display a wide range of alterations to root and shoot development (Buer *et al.,* 2009). Flavonoids have recently been implicated in the anti-venom protease activity of some Nigerian tropical plants (Ibrahim *et al.,* 2011). These large groups of compounds serve as UV protectants (Schmelzer *et al.,* 1988), signal molecules in plant microbe interactions (Long, 1989) and antibiotics in plant defence responses (Lamb *et al.,* 1989). Flavonoids carry out antioxidant action through scavenging or chelating process and are reported to play a preventive role in cancer and heart disease (Middleton *et al.,* 2000). Flavonoids and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing fever and pain (Baumann *et al.,* 1980). Flavonoids have a protective role during drought stress (Tattini *et al.,* 2004) and help plants to live on soils rich in toxic metals such as aluminium (Barcel´o and Poschenrieder 2002). Epicatechin, quercetin and luteolin types of flavonoids inhibit the development of fluids that result in diarrhoea by targeting intestinal cystic fibrosis membrane conductance regulators (Schuier *et al.,* 2005). Flavonoid content in leaves and flowers assessed as 22.82 ± 0.32 and 22.40 ± 0.20 µg of quercetin equivalents g-1 of extract correspondingly. The outcomes acquired were presented in fig. 1 (b).

Tannins are a group of polymeric phenolic substances capable of tanning leather or precipitating gelatic from solution (Scalbert 1991), causing local tumours (Kapadia *et al.,* 1978), inactivating and killing microorganisms. Some tannins are also known to have strong anticarcinogenic, antioxidant activities (Hausteen, 2005) and anti -HIV agent (Sayeed, 2007). Tannins are reported to exhibit antiviral, antibacterial and antitumor activities and also used as diuretic (Aiyelaagbe and Osamudiamen 2009). Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues and also used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003). Tannins complexes proteins, divalents metals, cellulose, hemicellulose, pectin and other carbohydrates (Mahanato *et al.,* 1982). Tannin content of *Leucas indica* leaves and flowers was 1.56 ± 0.02 and 1.15 ± 0.02 µg of tannic acid equivalents g -1 of extract correspondingly, results were put on show in figure: 1(c).

Table: 1 (d): Total alkaloid content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg of boldine equivalents/gram of extract	Flowers µg of boldine equivalents/gram of extract
25	124 ± 4	159 ± 5
50	255 ± 5	291 ± 9
100	392 ± 5	495 ±8

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	107488.222	2	53744.111	2.325E3	.000
	Within Groups	138.667	6	23.111		
	Total	107626.889	8			
Flowers	Between Groups	171624.222	2	85812.111	1.560E3	.000
	Within Groups	330.000	6	55.000		
	Total	171954.222	8			

Each value represents the mean±SD of three replicates

P<0.05 was considered as significant difference

Table: 1(e): Total saponin content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration Mg	Leaves µg/gram of extract	Flowers µg/gram of extract
25	5.15 ± 0.57	4.63 ± 0.55
50	9.66 ± 0.41	7.87 ± 0.21
100	13.07 ± 0.53	11.27 ± 0.26

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Leaves	Between Groups	94.619	2	47.310	181.975	.000
	Within Groups	1.560	6	.260		
	Total	96.179	8			
Flowers	Between Groups	66.147	2	33.074	240.419	.000
	Within Groups	.825	6	.138		
	Total	66.973	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

Table: 1 (f): Ascorbate content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg/gram of extract	Flowers µg/gram of extract
25	0.158 ± 0.004	0.152 ± 0.007
50	0.295 ± 0.015	0.292 ± 0.012
100	0.481 ± 0.022	0.454 ± 0.015

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Leaves	Between Groups	.157	2	.079	330.269	.000
	Within Groups	.001	6	.000		
	Total	.159	8			
Flowers	Between Groups	.137	2	.068	525.021	.000
	Within Groups	.001	6	.000		
	Total	.138	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

Alkaloid is one of the most diverse groups of secondary metabolites found in living organisms with a wide array of biosynthetic pathways, structural types and even pharmacological activities (Roberts and wink 1998). Recently, alkaloids obtained from plants have been reported to have antiangiogenic activity and these alkaloids may act through different mechanisms to inhibit angiogenesis (Flavia *et al.,* 2013). The biological function of alkaloids is very important and is used in analgesic, antispasmodic and bactericidal activities. Morphine, quinine, ephedrine, nicotine and strychnine are the major types of alkaloids some of these are nacrotic analgesics as well as are anti-tissue agent. Our outcomes specifically alkaloid content of leaves and flowers was 392 ± 5 and 495 ±8 of boldine equivalents g-1of extract respectively. Tabular and graphical representations were in figure: 1(d).

Saponins in *Leucas indica* leaves and flowers are ascertained, as they are glycoside components often referred to as "natural detergent" because of their foamy nature (Seigler, 1998). Saponins have been known to posses both beneficial and deleterious properties depending on its concentration in the

sample (Seigler, 1998). Seigler (1998) reported that saponins have anticarcinogenic properties, immune modulation activities and regulation of cell proliferation as well as health benefits such as inhibition of growth of cancer cells and cholesterol lowering activity. Saponins have been found to be potentially useful for the treatment of hyperglycaemia (Olaleye, 2007). Saponins inhibit Na^+ efflux by the lockage of the entrance of the Na^+ out of the cell. This leads to higher Na^+ concentration in the cells, activating a Na^+-Ca^{2+} anti porter in cardiac muscle. The increase in Ca^{2+} influx through this anti porter strengthens the contractions of heart muscle (Schneider and Woliling, 2004). Antifungal activity of some saponins has been reported (Khan and Srivastava 2009). The estimated saponins in *Leucas indica* leaves and flowers were 13.07 ±0.53 and 11.27 ± 0.26 µg/gram of extract dry matter respectively. Results were put on show in figure: 1(e).

Table: 1 (g): β-Carotene content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg/gram of extract	Flowers µg/gram of extract
25	13.42 ± 0.41	4.57 ± 0.33
50	25.86 ± 0.17	10.41 ± 0.40
100	41.22 ± 0.23	14.89 ± 0.17

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Leaves	Between Groups	1163.523	2	581.762	7.072E3	.000
	Within Groups	.494	6	.082		
	Total	1164.017	8			
Flowers	Between Groups	160.472	2	80.236	813.020	.000
	Within Groups	.592	6	.099		
	Total	161.064	8			

Each value represents the mean±SD of three replicates

$P<0.05$ was considered as significant difference

Table: 1 (h): Lycopene content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg/gram of extract	Flowers µg/gram of extract
25	9.07 ± 0.04	1.79 ± 0.19
50	17.74 ± 0.22	3.47 ± 0.25
100	28.59 ± 0.51	5.54 ± 0.25

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	573.936	2	286.968	2.763E3	.000
	Within Groups	.623	6	.104		
	Total	574.560	8			
Flowers	Between Groups	21.201	2	10.600	202.988	.000
	Within Groups	.313	6	.052		
	Total	21.514	8			

Each value represents the mean±SD of three replicates

$P<0.05$ was considered as significant difference

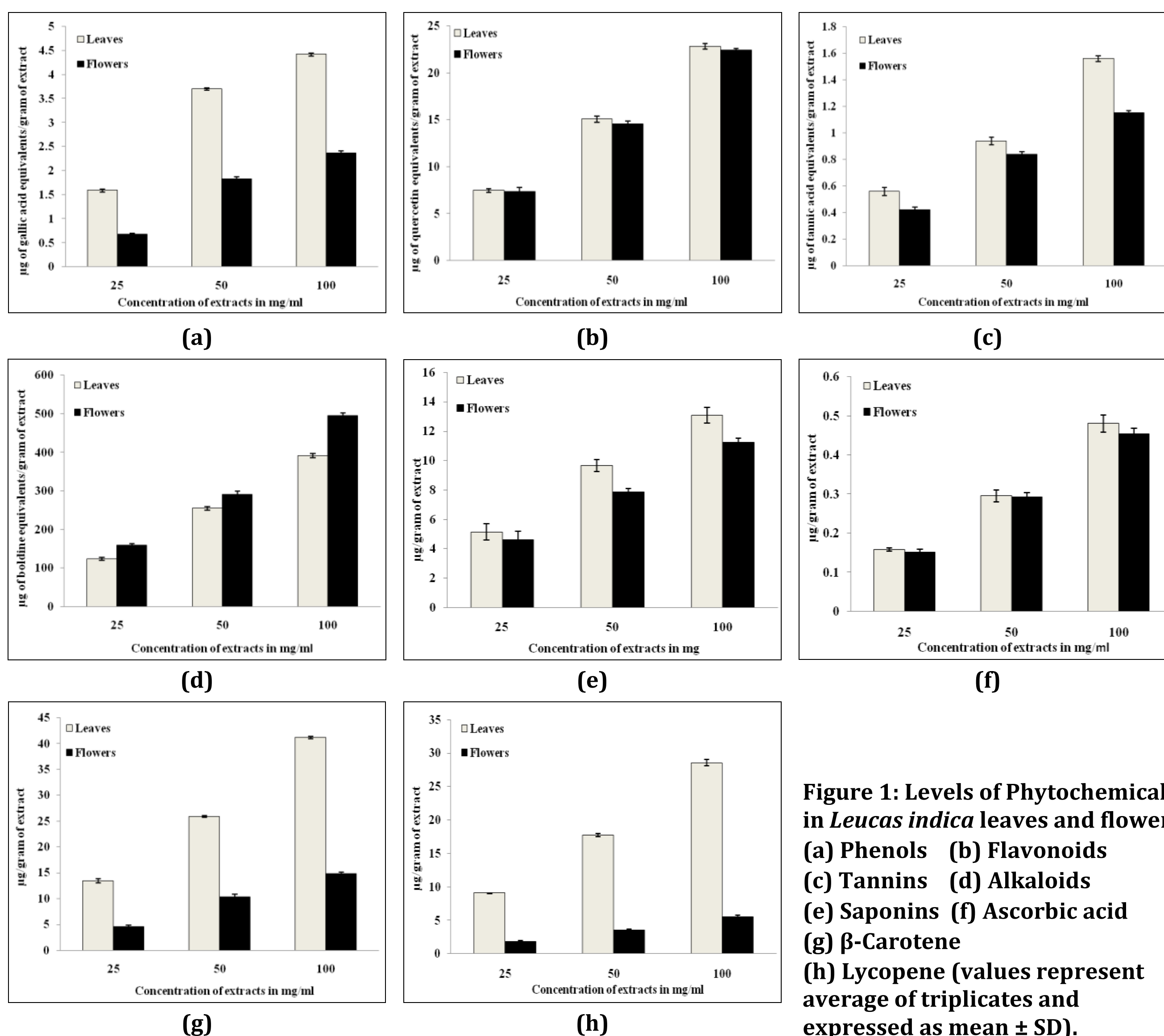


Figure 1: Levels of Phytochemicals in *Leucas indica* leaves and flowers (a) Phenols (b) Flavonoids (c) Tannins (d) Alkaloids (e) Saponins (f) Ascorbic acid (g) β-Carotene (h) Lycopene (values represent average of triplicates and expressed as mean ± SD).

Natural ascorbic acid is vital for the body performance (Aiyelaagbe and Osamudiamen, 2009). Vitamin C is the most important vitamin for human nutrition that is supplied by fruits and vegetables. L-Ascorbic acid (AA) is the main biologically active form of vitamin C. AA is reversibly oxidized to form L-dehydroascorbic acid (DHA), which also exhibits biological activity. Ascorbic acid provides first line of defence against oxidative stress (Nicolas Smirnoff 1996). The concentration of ascorbic acid in *Leucas indica* leaves and flowers was 0.481 ± 0.022 and 0.454 ± 0.015 µg/gram of extract respectively. Results were put on show in figure: 1(f).

The dietary carotenoids serve as precursor for vitamin A and prevent several chronic-degenerative diseases. Carotenoids have been extensively studied in different matrices to analyze their distribution and levels, as diet rich in carotenoids imparts health benefit properties. They are the most widely distributed pigments in nature (Gupta *et al.,* 2015). Lycopene is a vibrant red carotenoid that serves as an intermediate for the biosynthesis of other carotenoids and is found in moderate to high concentrations in foods such as tomato, watermelon, red grapefruit, and Brazilian guava (Stahl and Sies, 1996). Like its biosynthetic derivatives such as β-carotene, lycopene is an efficacious free radical scavenger (DiMascio *et al.,* 1989) and its presence in the diet positively correlates with reduced cancer incidence (Rao and Agarwal, 1998). Carotenoids and Lycopene contents of *Leucas indica* leaves and flowers were 41.22 ± 0.23, 14.89 ± 0.17 µg/gram of extract and 28.59 ± 0.51, 5.54 ± 0.25 µg/gram of extract correspondingly; results were put on show in figures: 1(g) and (h). From the above consequences, it evidently exposed that methanolic leave extract had elevated phytochemical constituents than methanolic flower extract, apart from alkaloids.

Antioxidant capacity assays

Antioxidants are radical scavengers which protects the human body from the pathological effects of free radicals. These chemical compounds are found in various processed foods or medicines as preservatives. But the increasing concern about chemical toxicity of the synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has triggered public interest in naturally derived antioxidants from diet, supplements and medicinal plants. The reducing ability of plant extracts may be indicators of their antioxidant potentials (Oyaizu 1986). The presence of phenolic antioxidants causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form which indicates higher reducing ability. Our results suggest that *Leucas indica* methanolic leave and flower extracts had high electron donating capacity as well as strong redox potential and can act as a reducing agent in quenching free radicals.

Table: 2 (a): DPPH activities in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves % of inhibition/50µl of extract	Flowers % of inhibition/50µl of extract	RUTIN % of inhibition/50µl of Rutin	BHT % of inhibition/50µl of BHT
25	22.92±0.12	18.18 ±0.42		
50	40.36±0.23	31.45±0.41		
100	61.17±0.16	50.22±0.25		
1			46	75

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	2201.037	2	1100.519	3.517E4	.000
	Within Groups	.188	6	.031		
	Total	2201.225	8			
Flowers	Between Groups	1554.967	2	777.484	5.643E3	.000
	Within Groups	.827	6	.138		
	Total	1555.794	8			

Each value represents the mean±SD of three replicates

P<0.05 was considered as significant difference

Table:2 (b): FRAP activities in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves FRAP Units	Flowers FRAP Units
25	32.52±0.44	25.35±0.31
50	64.34±0.41	49.20±0.23
100	98.11±0.30	78.21±0.31

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	6452.999	2	3226.500	2.121E4	.000
	Within Groups	.913	6	.152		
	Total	6453.912	8			
Flowers	Between Groups	4205.094	2	2102.547	2.608E4	.000
	Within Groups	.484	6	.081		
	Total	4205.577	8			

Each value represents the mean±SD of three replicates

P<0.05 was considered as significant difference

Table: 2 (c): Reducing power assay of methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg of Ascorbic acid equivalents/1ml of extract	Flowers µg of Ascorbic acid equivalents /1ml of extract
25	15.51±0.19	11.51±0.19
50	30.14±0.12	18.62±0.20
100	58.16±0.15	40.56±0.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	2818.091	2	1409.045	5.857E4	.000
	Within Groups	.144	6	.024		
	Total	2818.235	8			
Flowers	Between Groups	1375.138	2	687.569	1.710E4	.000
	Within Groups	.241	6	.040		
	Total	1375.380	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

Table: 2 (d): Hydroxyl Radical Scavenging activities of methanolic extracts of leaves and flowers of *Leucas indica*

Concentrat ion mg/ml	Leaves % of inhibition/ 0.1 ml of extract	Flowers % of inhibition/0.1 ml of extract	Ascorbic acid % of inhibition/0.1 ml of ascorbic acid	BHT % of inhibition / 0.1 ml of BHT
25	16.46±0.21	10.19±0.19		
50	27.67±0.29	20.47±0.24		
100	53.32±0.17	36.43±0.21		
1			73	73.6

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	2142.246	2	1071.123	2.095E4	.000
	Within Groups	.307	6	.051		
	Total	2142.553	8			
Flowers	Between Groups	1048.900	2	524.450	1.164E4	.000
	Within Groups	.270	6	.045		
	Total	1049.170	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

The extracts under study demonstrated superb free radical scavenging activity that is similar to standard antioxidants at all concentration tested. High antioxidant activity of plant extracts has previously been attributed to the phenolic compounds (Odagbasoglu *et al.,* 2004) and flavonoids, saponins and tannins (Aliyu *et al.,* 2009). Free-radical scavengers are antioxidants which can provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and subsequent lipid peroxidation, protein damage and DNA strand breaking (Palanisamy and Sellappa 2012).

Table: 2 (e): ABTS activities in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves TEAC50	Flowers TEAC50
25	32.27±0.25	25.26 ±0.31
50	64.56±0.23	48.91 ±0.33
100	97.77±0.23	77.91 ±0.24

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	6436.456	2	3218.228	5.754E4	.000
	Within Groups	.336	6	.056		
	Total	6436.792	8			
Flowers	Between Groups	4172.345	2	2086.173	2.367E4	.000
	Within Groups	.529	6	.088		
	Total	4172.874	8			

Each value represents the mean±SD of three replicates
$P<0.05$ was considered as significant difference

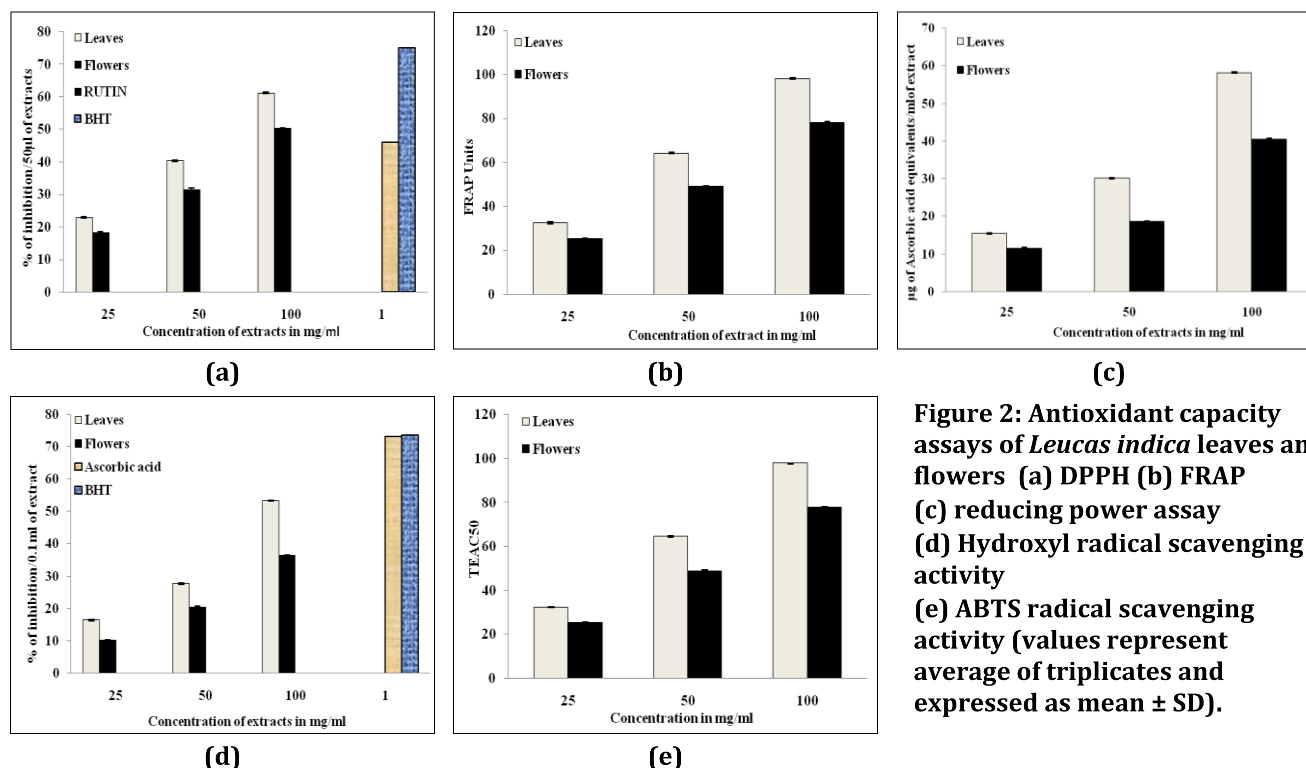


Figure 2: Antioxidant capacity assays of *Leucas indica* leaves and flowers (a) DPPH (b) FRAP (c) reducing power assay (d) Hydroxyl radical scavenging activity (e) ABTS radical scavenging activity (values represent average of triplicates and expressed as mean ± SD).

The over production of ROS has implicated in more than 100 degenerative diseases including heart diseases, atherosclerosis, diabetes, cancer etc (Nunomura *et al.*, 2006). Free radicals act as a trigger to a number of degenerative diseases. Therefore, samples having free radical scavenging activity can be of potent medicinal importance (Chen and Ho, 1995). DPPH is commonly used as a tool to evaluate the free radical scavenging activity of new compounds (Muchuweti *et al.*, 2007). Reducing power assay is

another convenient and rapid screening method for measuring the antioxidant potential (Chanda *et al.,* 2011). In addition, reducing power of a compound is related to electron transfer ability of the compound which could lead to the neutralization of free radicals (Zhu *et al.,* 2001). The FRAP assay is based on the ability of a sample to reduce Fe3+ in a Tripyridyltriazine (TPTZ) solution to Fe^{2+} and create the blue-coloured complex Fe^{2+}– TPTZ. Increased concentrations of the above complex means an increased FRAP value. Hydroxyl radicals are one of the quick initiators of the lipid peroxidation process by abstracting hydrogen atom from unsaturated fatty acids or simply auto oxidation of polyunsaturated fatty acids found primarily in membranes. Reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity (SerbetciTohma and Gulcin 2010)

Therefore, antioxidant capacities were ascertained in *leucas indica* leaves and flowers, they were increased in a dose dependent manner ranged from 25 – 100 mg/ml. The outcomes of antioxidant capacity assays mentioned below were at 100 mg/ml. DPPH radical scavenging activity of leaves and flowers was 61.17±0.16 and 50.22±0.25 % of inhibition/50µl of extract as compared to 46 and 75% of inhibition per 1 mg/ml of Rutin and BHT as positive controls. For ferric reducing ability power (FRAP), the outcome observed as 98.11 ±0.30 and 78.21±0.31 FRAP Units for leaves and flowers correspondingly. In favour of reducing power assay, results were observed as 58.16±0.15 and 40.56±0.21 µg of ascorbic acid equivalents per ml of extract for leaves and flowers in that order. Hydroxyl Radical Scavenging activity outcomes were 53.32±0.17 and 36.43±0.21 % of inhibition per 0.1 ml of extract for leaves and flowers of *leucas indica*, as compared to the results of Positive controls ascorbic acid and BHT at 1 mg/ml with percentage of inhibition 73 and 73.6 in that order. Finally, in ABTS method, the highest TAC value was observed in leaves (97.77±0.23 TEAC50) and the less value was observed in leaves (77.91 ±0.24 TEAC50), Previously, Paweł Pas´ko *et al.,* (2009) estimated ABTS in amaranth and quinoa seeds and sprouts during their growth. Penarrieta *et al.,* (2008) made a comparitive study of ABTS in between pseudocereals and cereals, There was a strong correlation between ABTS and DPPH, which was also observed previously (Awika *et al.,* 2003). All the above results agree with the preceding information and outcomes were portrayed in Figures: 2 (a), (b), (c), (d) and (e). correspondingly. Velioglu *et al.,* (1998) worked out on antioxidant activity and total phenolics in selected fruits vegetables and grain products. All the acquired results are in line with preceding information. From the above fallouts, it clearly reveeld that methanolic leave extract showed higher antioxidant capacity compared with methanolic flower extract, this may be due the presence of high phytochemical constituents.

CONCLUSION

In view of the consequences obtained from phytochemical screening and antioxidant activities of *Leucas indica* leave and flower methanol extracts contains promising antioxidant and phytochemical ingredients and that might be responsible for the therapeutic activities of the plant aerial part extracts in the treatment of various diseases. Further work to isolate and characterize the organic constituents as well as toxicological studies may enhance the understanding or the scientific basis of its uses in traditional medicine.

REFERENCES

Kumar T and Chandrashekar KS (2011) *Bauhinia purpurea Linn.:* A review of its ethnobotany, phytochemical and pharmacological profile. *Res J Med Plant.* 5: 420- 431.

Samy RP, Pushparaj PN and Gopalakrishnakone P (2008) A compilation of bioactive compounds from ayurveda. *Bioinformation.* 3:100-110.

Sharma Iti (2013) Arsenic-induced oxidative stress and antioxidant defence system of *Pisum sativum* and Pennisetum typhoides: Acomparative study research. *J Biotechnol.* 8:48-56.

Chen CW and Ho CT (1995) Antioxidant properties of polyphenols extracted from green and black tea. *J Food Lipids.* 2: 35-46.

Madhava Chetty K, Sivaji K, Tulasirao K (2008) Flowering plants of Chittor Distric, Andhra Pradesh. Turupathi; India Students offset printers; 277.

Javanmardi J, Stushnoff C, locke E and Vivanco JM (2003) Antioxidant activity and total phenolic content of Iranian Ocimum accessions*. Food Chem*. 83: 547-550.

Bao J, Cay Y, Sun M, Warg G and Corke H (2005) Anthocyanins, flavonol and free radical scavenging activity of Chinese bayberry (Myrica rubra) extracts and their color properties and stability. *J Agric Food Chem*. 53: 2327-2332.

Folin O and Ciocalteu V (1927) On tyrosine and tryptophane determinations in proteins. *J Biol Chem.* 73: 627–650.

Sreevidya N and Mehrotra S (2003) Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. *J AOAC Int.* 86: 1124–1127.

Obdoni BO and Ochuko PO (2001) "Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria" *Global J Pure Appl Sci.* 8: 203-208.

Nagata M and Yamashita I (1992) Simple method for simultaneous deter mination of chlorophyll and carotenoids in tomato fruit. *Nippon Shokuhin Kogyo Gakkaish.* 39(10), 925–928.

Sadasivam S and Theymoli B (1987) Practical Manual in Biochemistry: Tamilnadu Agriculture University; Coimbattore. 14.

Re R, Pelledrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radicals in Biology and Medicine.,* 26; 1231–1237.

Barton H, Fołta M and Zachwieja Z (2005) Application of FRAP, ABTS and DPPH methods to estimation of antiopxidant activity of food products. Nowiny Lekarskie., 4; 510–513 [in Polish; English abstract].

Cuendet M, Hostettmann K and Potterat O (1997) Iridoidglucosides with free scavenging properties from Fagracablumei. *Helv Chim Acta.* 80:1144 -1152.

Benzie IFF and Strain JJ (1996) Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal Biochem.* 239: 70-76.

Oyaizu M (1986) Studies on product of browning reaction prepared from glucose amine. *Jap J Nutr.* 44: 307-315.

Gulhan VU, ferda C, Atalay S, Dimitra D, Moschos P, Munevver S, Erol D and Bektas T (2003) Antimicrobial and Antioxidant Activity of the Essential Oil and Methanol Extracts of Thymus pectinatus Fisch. et Mey. Var. pectinatus (Lamiaceae). *J Agric Food Chem.* 51: 63-67.

Dixon RA and Paiva NL (1995) Stress-induced phenylpropanoid metabolism. *Plant Cell.* 7: 1085-1097.

Lynn DG and Chang M (1990) Phenolic signals in cohabitation: implications for plant development. *Annu Rev Plant Physiol Plant Mol Biol.* 41: 497-526.

Mamta Saxena, Jyoti Saxena and Alka Pradhan (2012) Flavonoids and phenolic acids as antioxidants in plants and human health. Int J Pharm Sci Rev and Res. 16:130-134.

Shirley BW (1998) Flavonoids in seeds and grains: physiological function, agronomic importance and the genetics of biosynthesis. *Seed Sci Res.* 8: 415- 422.

Buer CS and Djordjevic MA (2009) Architectural phenotypes in the transparent testa mutants of Arabidopsis thaliana. *J Exp Bot.* 60: 751–763.

Ibrahim MA, Aliyu AB, Abusufiyanu A, Bashir M and Sallau AB (2011) Inhibition of Naja nigricolis (Reinhardt) venom protease activity by Luffa egyptiaca (Mill) and Nicotiana rustica (Linn) extracts. *Indian J Exp Biol.,* 49(7): 552-554.

Schmelzer E., Jahnen W and Hahlbrock K (1988). In situ localization of light-induced chalcone synthase mRNA, chalcone synthase and flavonoid end products in epidermal cells of parsley leaves. *Proc Natl Acad Sci.* 85: 2984-2993.

Long S (1989) Rhizobium-legume nodulation: Life together in the underground. Cell. 56: 203-214.

Lamb CJ, Lawton MA, Dron M and Dixon RA (1989) Signals and transduction mechanisms for activation of plant defences against microbial attack. *Cell.* 56: 215-224.

Middleton EJr, Kandaswami C and Theoharides TC (2000) The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease cancer. *Pharmacol Rev.* 52: 673-751

Baumann J, Von Brucchau SV and Wurm G (1980) Flavonoids and related compounds as inhibition of arachidonic acid peroxidation. *Prostaglandins.* 20:627–639.

Tattini M, Galardi C, Pinelli P, Massai R, Remorini D, Agati G (2004) Differential acumulation of flavonoids and hydroxycinnamates in leaves of Ligustrum vulgare under excess light and drought stress. *New Phytol.* 163:547–561.

Barcel´o J, Poschenrieder C (2002) Fast root growth responses, root exudates, and internal detoxification as clues to the mechanism of aluminium toxicity and resistance: *a review Environ Exp Bot*. 48:75–92.

Schuier M, Sies H, Billek B, Fischer H (2005) Cocoa-Related Flavonoids Inhibit CFTR-Mediated Chloride Transport across T84 Human Colon Epithelia. *J Nutr.* 135: 2320-2325.

Scalbert A (1991) Antimicrobial properties of tannins. *Phytochem.* 30: 3875-3883.

Kapadia G, Chung E, Ghosh B, Shukla Y, Basak S, Morton J and Pradhan S (1978) Carcinogenicity of some folk medicinal herbs in rats. *J Natl Cancer Inst.* 60: 683-686.

Hausteen BH (2005) The Biochemistry and medical significance of the flavonoids. *Pharmacol therapeutics J*. 96: 67-202.

Sayeed Ahmad (2007) Introduction of plant constituents and their tests. *Pharmacognosy*

Aiyelaagbe OO and Osamudiamen PM (2009) Phytochemical screening for active compounds in Mangifera indica leaves from Ibadan, Oyo State. *Plant Sci Res.* 2: 11-13.

Parekh J and Chanda S (2007) In vitro antibacterial activity of crude methanol extract of Woodfordia fruticosa Kurz flower (Lythacease). *Braz J Microbiol.* 38: 204-207.

Dharmananda S (2003) Gallnuts and the uses of Tannins in Chinese Medicine. In: Proceedings of Institute for Traditional Medicine, Portland, Oregon.

Mahanato SB, Ganguly AN, Sadhu NP (1982) Review – steroid saponins. *Phytochem.* 21(5):959-978.

Roberts MF and Wink M (1998) Introduction In: Alkaloids: Biochemistry, Ecology and Medicinal Applications, M.F.Roberts & M. Wink, pp. 1-6, Plenum Press, ISBN 0-306- 45465-3, New York.

Flavia Cristine Mascia Lopes, Luis Octavio Regasini, Manueal Nuno de Magalhaes Pinheiro Alcada, Raquel Soares (2013) Antiangiogenic Alkaloids from plants. *Nat Prod.* 67:1439-1467.

Seigler DS (1998) Plant Secondary Metabolism. Dordrecht: Kuwar Academic Press.

Olaleye MT, (2007) Cytotoxicity and antibacterial activityof methanolic extract of *Hibiscus sabdariffa.* J. Med.Plants Res., 1: 9-13.

Schneider G and Woliling J (2004) Synthetic Cardenolides and Related compounds. Current Organic Chemistry, pp 8.

Nicolas Smirnoff (1996) The functional metabolism of ascorbic acid in plants.*Annals Bot.* 78: 661-669.

Gupta, Yellamaraju Sreelakshmi and Rameshwar Sharma (2015) A rapid and sensitive method for determination of carotenoids in plant tissues by high performance liquid chromatography. *Plant Methods*.11:5

Stahl W and Sies H (1996) Lycopene: a biologically important carotenoid for humans? *Arch BiochemBiophys*. 336: 1–9.

DiMascio P, Aaiser S and Sies H (1989) Lycopene as the most effective biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys.* 274: 532–538.

Rao AV and Agarwal S (1998) Bioavailability and in vitro antioxidant properties of lycopene fromtomato products and their possible role in the prevention of cancer.Nutr. Cancer 31, 199–203.

Odabasoglu F, Asian A, Cakir A, Suleyman H and Karagoz Y (2004) Comparison of antioxidant activity and total phenolics content of three lichen species.*Phytotherapy Res*. 18: 938-941.

Aliyu MS, Hanwa UA, Tijjani MB, Aliyu AB and Ya'u B (2009) Phytochemical and antibacterial properties of leaf extract of *Stereospermum kunthianum* (Bignoniaceae). *Nigerian Journal of Basic and Applied Sciences*, **17(2):** 235-239.

Palanisamy Senthil Kumar and Sellappa Sudha (2012) Evaluation of antioxidant activity and total phenolic content of *Padina boergesenii* from Gulf of Mannar. *Drug Inven Today*. 4:635-63.

Nunomura A, Castellani RJ, Zhu X, Moreira PI, Perry G and Smith MA (2006) Involvement of oxidative stress in Alzheimer disease. *J NeuropatholExp Neurol.* 65: 631–641

Muchuweti M, Mupure C, Ndhlala A, Murenje T and Benhura MAN (2007) Screening of antioxidant and radical scavenging activity of *Vigna ungiculata*, *Bidens pilosa* and *Cleome gynandra*. *Am J Food Technol.* 2: 161-168

Chanda S, Kaneria M and Nair R (2011) Antibacterial Activity of *Psoralea corylifolia L*. Seed and Aerial Parts with Various Extraction Methods. *Res J Microbiol.* 6: 124-131.

Zhu N, Wang M, Wei GJ, Lin JK, Yang CS, et al. (2001) Identification of reaction products of (−)*Epigallocatechin*, (−)-*Epigallocatechin gallate* and pyrogallol with 2,2-diphenyl-1-picrylhydrazylradical. Food Chemistry 73: 345- 349.

Serbetci Tohma H, Gulçin I (2010) Antioxidant and radical scavenging activity of aerial parts and roots of Turkish liquorice (*Glycyrrhiza glabra L*.). International Journal of Food Properties 13: 657-671.

Pawel Pasko, Henryk Barton, Paweł Zagrodzki, Shela Gorinstein, Maria Fołta, Zofia Zachwieja (2009) Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. *Food Chemistry.,* 115: 994–998.

Penarrieta JM, Alvarado JA, Akesson B and Bergenstahl B (2008) Total antioxidant capacity and content of flavonoids and other phenolic compoundsin canihua *Chenopodium pallidicaul* An Andean pseudo cereal. MolecularNutrition & Food Research, 52(6), 708–717.

Awika JM, Rooney LW, Wu X, Prior RL, Cisneros-Zevallos, L (2003) Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. J Agric Food Chem 51: 6657–6662.

Velioglu YS, Mazza G, Gao L and Oomah BO (1998) Antioxidant activity and total phenolics in selected fruits vegetables and grain products. *J Agric Food Chem.* 46: 4113-4117.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

An evaluation of comparative biosorption study of Cadmium (II) and Chromium (VI) using Orange rind (*Citrus sinensis*), (L.) Osbeck, under optimized conditions

Poojari Anukthi C[1], Maind Sandip D[2] and Bhalerao Satish A[1*]

[1]Environmental Sciences Research Laboratory, Department of Botany, Wilson College, Mumbai-400007,
[2]Department of Chemistry, Bharatiya Vidya Bhavan's Hazarimal Somani College of Arts and Science, Kulapati K.M. Munshi Marg, Chowpatty, Mumbai-400007, Maharashtra, India
*Corresponding Author Email : drsatishbhalerao55@gmail.com | drsatish16@rediffmail.com

Manuscript details:

Received: 15.02.2016
Revised : 24.03.2016
Accepted: 26.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Poojari Anukthi C, Maind Sandip D and Bhalerao Satish A (2016) An evaluation of comparative biosorption study of cadmium (II) and chromium (VI) using orange rind (*Citrus sinensis*), (L.) Osbeck,under optimized conditions. *International J. of Life Sciences*, 4(1): 44-56.

Acknowledgements
The authors are thankful to Principal Dr. V. J. Sirwaiya for their administrative support, cooperation and help. Thanks to Viva College for FTIR analysis and IIT, Mumbai for SEM analysis.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

The present study investigated the comparative biosorption study of cadmium (II) and chromium (VI) using inexpensive biosorbentrind of orange (*Citrus sinensis*), (L.) Osbeck, under optimized conditions. A biosorption study was carried out in batch system from aqueous solutions. The biosorbent before and after biosorption was characterized by FTIR and SEM. The work considered the optimization of parameters such as solution pH, biosorbent dose, initial metal concentration, contact time and temperature. To assess the potential applicability of biosorbent, the experimental equilibrium data were analysed by Langmuir, Freundlich, Dubinin-Kaganer-Redushkevich (DKR) and Temkin isotherms. Langmuir isotherm model provided a better fit with the experimental data for both cadmium (II) and chromium (VI). The maximum biosorption capacity of cadmium (II) and chromium (VI) which was determined from Langmuir isotherm was found to be 83.33mgg^{-1}and 10.74mg g^{-1}respectively. Simple kinetic models such as pseudo-first-order, pseudo-second-order, Elovich and Weber & Morris intraparticle rate diffusion, were employed to determine the biosorption mechanism. A result clearly indicates that the pseudo-second-order kinetic model was found to be correlating the experimental data strongest for both cadmium (II) and chromium (VI), which suggests that chemical adsorption process was more dominant. Thermodynamic study revealed that the biosorption process was spontaneous, endothermic and increasing randomness of the solid solution interfaces. The rind of orange (*Citrus sinensis*), (L.) Osbeck was found to remove cadmium (II) and chromium (VI) effective from aqueous solutions with uptake and selectivity in the order of cadmium (II) > chromium (VI).Thus biosorption have an advantages over other expensive cleanup technologies, can be used for waste water treatment for remediation of heavy metal contamination in industrial sectors.

Keywords: *Comparative study, Biosorption, Cadmium (II), Chromium. (VI), Orange (Citrus sinensis), (L.) Osbeck, FTIR, SEM, Adsorption isotherms, Adsorption kinetics, Thermodynamic study.*

INTRODUCTION

Heavy metal pollution of waste water, as a result of enormous industrial development and modernization is one of the most common environmental problems. Heavy metals are carcinogenic properties (Cimino and Caristi, 1990) and recalcitrant even at very low concentrations & they can pollute drinking water resources. Heavy metals (cadmium and chromium) are highly toxic, non-biodegrable and can accumulate along the food chain which results in serious ecological and health hazard. Cadmium (II) finds its way into water bodies through industries like metal production, phosphate fertilizers, pesticides, electroplating, textile operations, manufacture of batteries and pigments & dyes (Sharma, 2008; Perez-Marin *et al.*, 2007). Chromium (VI) discharge into the environment can be due to various large numbers of industrial functions like dyes and pigments production, film and photography, galvanometry, metal cleaning, plating and electroplating, leather and mining, etc.(Patterson, 1985).Cadmium (II) causes sterility and is harmful to human health. Cadmium (II) is likely to cause a number of acute and chronic disorders, such as itai-itai disease, renal damage, emphysema, hypertension, testicular atrophy, damage to the kidneys, lungs & liver, carcinogenesis etc. Diseases caused by chromium (VI) are bronchial asthma and lung cancer. When heavy metals (cadmium and chromium) are present in the wastewater beyond the permissible limits of concentration, it can have severe toxicological effects on both human and aquatic ecosystems. United States Environmental Protection Agency (USEPA) and World Health Organization (WHO) have demarcated the permissible limit of cadmium and chromium in potable water as 0.003 mg/L and 0.05mg/L respectively (WHO, 2008). Hence, the removal of heavy metals becomes mandatory before the discharge of industrial effluents into main water stream.

The conventional methods for removing heavy metal ions from industrial effluents include oxidation/ reduction, filtration by membranes, chemical precipitation, coagulation, solvent extraction, cementation, freeze separation, reverse osmosis, ion-exchange, electro-dialysis, electro-winning and electro-coagulation (Ahluwalia and Goyal, 2007). These methods have found limited application because they often involve high capital and operational cost. Treatment of industrial effluent with sorbents of biological origin is simple, comparatively inexpensive and friendly to the environment. Biosorption of heavy metals is very effective, versatile, powerful, most efficient and cost effective technologies involved in the removal of heavy metals from industrial effluents (Bhalerao, 2011). Biosorption is the process based on the principle of metal binding capacities of biological materials. Biosorption is a process that utilizes low-cost biosorbents to sequester toxic heavy metals. Biosorption has distinct advantages over expensive clean up technologies which used in industrial sector. The major advantages of biosorption which include reusability of biomaterial, low operating cost, high efficiency of metal removal from dilute solution, no additional nutrient requirement, short operation time, no chemical and/or biological sludge and the possibility of metal recovery (Kratochvil and Volesky, 1998; Mungasavalli *et al.*, 2007).

In the recent years many low cost biosorbents materials have been utilized for heavy metal removal in waste water. We reported previously and investigations have been carried out to identify suitable and relatively cheap biosorbents that are capable of removing significant quantities of heavy metals ions (Maind *et al.*, 2012; Maind *et al.*, 2013; Maind *et al.*, 2012; Maind and Bhalerao 2013). Among the various resources in biological waste, both dead and live biomass, exhibit particularly interesting metal-binding capacities. The use of dead biomass eliminates the problem of toxicity and the economics aspects of nutrient supply and culture maintenance (Pino *et al.*, 2006). Many low cost biosorbents have been intensively examined for their abilities to be applied for removal of cadmium (II) and chromium (VI) from aqueous solutions.

The rind of orange (*Citrus sinensis*), (L.)Osbeck being one of the highest production fruit in Maharashtra state of India and in juice making industry produced large amounts of waste which has no commercial value. The rind of Orange (*Citrus sinensis*), (L.) Osbeck was selected because of a low cost, higher adsorption capacity, possibility of availability of function groups such as hydroxyl, carbonyl, carboxylic etc. which favours biosorption of heavy metals.

The aim of the present research wasto utilize the rind of orange (*Citrus sinensis*), (L.) Osbeck for the biosorption of cadmium (II) and chromium (VI) from aqueous solutions in a batch system. The objective of this study was to characterize biosorbent be four and

after biosorption using FTIR and SEM. The study was extended with the objective for estimation and calculation of various parameters affecting the biosorption of metals such as solution pH, biosorbent dose, contact time, initial metal concentration and temperature. Adsorption isotherms (Langmuir, Freundlich, Dubinin-kaganer-Redushkevich (DKR) and Temkin) and kinetics models (pseudo-first-order, pseudo-second-order, Elovich equation and intraparticlerate diffusion) was employed to understand the probable biosorption mechanism. Thermodynamic studies was also carried out to estimate the standard Gibbs free change (ΔG^0), standard enthalpy change (ΔH^0) and standard entropy change (ΔS^0).

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and reagents used were of analytical reagent (AR) grade. Double distilled water was used for all experimental work including the preparation of metal solutions. The desired pH of the metal ion solution was adjusted with the help of dilute hydrochloric acid and dilute sodium hydroxide.

Preparation of cadmium (II) chromium (VI) solution

The stock solution of 1000 ppm of cadmium (II) ions was prepared by dissolving 0.1g cadmium metal in 1 ml concentrated nitric acid and diluted in 250 ml of double distilled water. The stock solution of 1000 ppm of chromium (VI) was prepared by dissolving 0.7072 g of potassium dichromate ($K_2Cr_2O_7$) (AR grade) (previously dried at 50^0C for one hour) in 250 ml of double distilled water. Further desired test solutions of cadmium (II) chromium (VI) were prepared using appropriate subsequent dilutions of the stock solution.

Preparation of biosorbent

The oranges (*Citrus sinensis*), (L.)Osbeck was collected locally and the rind of oranges washed with several times with distilled water to remove the surface adhered particles, dirt, other unwanted material &water soluble impurities and water was squeezed out. The washed biosorbent was then dried at 50^0C overnight and grounded in a mechanical grinder to form a powder. The powder was sieved and a size fraction in the range of 100-200 µm will be used in all the experiments. This powder was soaked (20 g/l) in 0.1 M nitric acid for 1 hour. The mixture was filtered and the powder residue was washed with distilled water, several times to remove any acid contents. This filtered biomass was first dried, at room temperature and then in an oven at 105ºC for 1-2 hrs. For further use, the dried biomass was stored in air tighten plastic bottle to protect it from moisture.

Characterization of biosorbent by Fourier Transform Infrared (FTIR) analysis

The Fourier Transform Infrared (FTIR) spectroscopy was used to identify the functional groups present in the biosorbent. The biomass samples were examined using FTIR spectrometer (model:FT/IR-4100typeA) within range of 400-4000 cm^{-1}. All analysis was performed using KBr as back ground material. In order to form pellets, 0.02 g of biomass was mixed with 0.3 g KBr and pressed by applying pressure.

Characterization of biosorbent by Scanning Electron Microscope (SEM) analysis

The Scanning Electron Microscope (SEM) was used to see the porosity of the biosorbent. The samples were covered with a thin layer of gold and an electron acceleration voltage of 10 KV was applied and then Scanning Electron Micrograph was recorded.

Experimental procedure

The static (batch) method was employed at temperature (30^0C) to examine the biosorption of cadmium (II) and chromium (VI) by biosorbents. The method was used to determine the biosorption capacity, stability of biosorbent, and optimum biosorption conditions. The parameters were studied by combining biosorbent with solution of cadmium (II)and chromium (VI) in 250 ml separate reagent bottles. The reagent bottles were placed on a shaker with a constant speed and left to equilibrate. The samples were collected at predefined time intervals, centrifuged, the content was separated from the biosorbents by filtration, using Whatmann filter paper and amount of cadmium (II) and chromium in the supernatant/filtrate solutions was determined using digital UV-visible spectrophotometer (EQUIP-TRONICS, model no. Eq-820).The following equation was used to compute the percentage adsorption (% Ad) of cadmium (II) and chromium (VI) by the biosorbent,

$$\%Ad = \frac{(C_i - C_e)}{C_i} \times 100 \qquad (1)$$

Where C_i and C_e are the initial concentrations and equilibrium concentrations of the cadmium (II) and chromium (VI) in mg/L.

The equilibrium cadmium (II) and chromium (VI) adsorptive quantity was determined by the following equation,

$$q_e = \frac{(Ci - Ce)}{w} \times V \qquad (2)$$

Where q_e (mg metal per g dry biosorbent) is the amount of cadmium (II) or chromium (VI) adsorbed, V (in liter) is the solution volume and w (in gram) is the amount of dry biosorbent used.

Estimations of cadmium (II) and chromium (VI) concentration

Quantitative estimations of cadmium (II) and chromium were carried out by UV-visible spectrophotometer using dithizone and 1,5-Diphenylcarbazide as a complex forming regent for cadmium (II) and chromium (VI) respectively.

RESULTS AND DISCUSSION

Characterization of biosorbent by Fourier Transform Infrared (FTIR) analysis

To investigate the functional groups of biosorbent and metal loaded with biosorbent, a FTIR analysis was carried out and the spectra are shown in Fig.1. (a, b and c). As seen in the figure unloaded biomass displays a number of absorption peaks, reflecting the complex nature of biomass. The spectrums clearly showed the broad peak of -OH and -NH groups. The stretching of the –OH groups bound to methyl groups are clearly indicated in the spectrum. The characteristics peak of carbonyl group is present. The presence of -OH group along with carbonyl group confirms the presence of carboxyl acid groups in the biomass. The peak of stretching and the stretching in aromatic rings are present. The peaks of C-H and C-O bonds observed. The –OH, NH, carbonyl and carboxyl groups are important sorption sites (Volesky, 2003). As compared to simple biosorbent, biosorbent loaded with metal, the broadening of -OH group peak and carbonyl group peak was observed. This indicates the involvement of hydroxyl and carbonyl groups in the biosorption of metal.

Characterization of biosorbent by Scanning Electron Microscope (SEM) analysis

The surface characteristics, structure and particle size distribution of biosorbent before and after biosorption was examined using Scanning Electron Microscope (SEM). The SEM micrographs are shown in Fig. 2. (a, b and c). These micrographs represent a porous structure with large surface area. The SEM clearly demonstrated that there is more uniformity after biosorption on metal ions in comparison to before biosorption. It was evident from the mictrographs that the biosorbent presents an unequal structure before metal adsorbed. The number of canals in the biosorbent was higher in the initial case. The metal ions adsorbed on the cell wall matrix and created stronger cross linking and uniformity on the surface of biosorbent.

Effect of pH

pH is considered as a very important parameter in biosorption process. The functional groups responsible for binding of metal ions in the biosorbent, affected by pH. It also affects the competition of metal ions that gets adsorb to active sites of biosorbent. The biosorption capacity of the biosorbent and speciation of metals in the solution is pH dependent. pH influences the chemical structure of the cadmium (II) and chromium (VI) in aqueous solution, hence influencing its bioavailability. The biosorption capacity of the cadmium (II) and chromium (VI) depends on the pH of the biosorption medium, which influences electrostatic binding of cadmium (II) and chromium (VI) ions to corresponding functional groups. The optimization of pH was done by varying the pH in the range of 2 to 10 for cadmium (II) and 1 to 8 for chromium (VI) and pH trend observed in this case is shown in Fig. 3. It was found that biosorption of cadmium (II) with biosorbent has increased by increasing pH and at pH 7 the biosorption process was maximum with 80.30 % and then decreases till pH 10. The lesser biosorption at lower pH was due to lesser surface sites are available for biosorption. pH 7 was chosen for all further biosorption studies for cadmium (II). It was found that at pH 2, biosorption of chromium (VI) with biosorbent was maximum with 71.01 % and after increasing pH, biosorption was decreases. According to the solubility equilibrium of chromium, $HCrO_4^-$ is the dominant species of chrmoium (VI) at a pH 2. As the pH increases, the dominant form of chromium becomes CrO_4^{2-} and $Cr_2O_7^{2-}$. Furthermore, the surface of biosorbent may be positively charged at pH 2. Therefore, at this pH it is likely to bebiosorbed chrmoium (VI) onto biosorbent through electrostatic attraction and /or by the binding of $HCrO_4^-$ to acidic functional groups on the surface of biosorbent. Also at pH 2, the number of protons

available on the surface of biosorbent increases, which increases the attraction between $HCrO_4^-$ & biosorbent and increases the sorption capacity (Rao *et al.*, 1992). As the pH of the solution increases, charges on the surface of biosorbent becomes negative, this leads to generation of repulsive forces between chromium (VI) & biosorbent and inhibits biosorption and resultantly percent chromium(VI) uptake may decrease.

Effect of biosorbent dose

Effect of biosorbent dose of biosorption of metal ions onto biosorbent which is an important parameter was studied while conducting batch biosorption studies. The biosorption capacity of cadmium (II) and chromium (VI) ontothe rind of orange (*Citrus sinensis*), (L.)Osbeck by varying biosorbent dose from 1.0mg/ml to 20 mg/mlfor zinc (II) and 1.0 mg/ml to 15 mg/ml for chromium (VI) is as shown in Fig. 4. From the results it was found that biosorption of cadmium (II) and chromium (VI) increases with increase in biosorbent dose and is highly dependent on biosorbent concentration. Increase in biosorption by increase in biosorbent dose is because of increase of ion exchange site ability, surface areas and the number of available biosorption sites (Naiya *et al.*, 2009). The point of saturation for the rind of orange (*Citrus sinensis*), (L.) Osbeckwas found at 5 mg/mL of biosorbent dose with maximum removal efficiency for both cadmium (II) and chromium (VI). The decrease in efficiency at higher biosorbent concentration could be explained as a consequence of partial aggregation of biosorbent which results in a decrease in effective surface area for metal uptake (Karthikeyan *et al.*, 2007).The biosorbent dose 5mg/ml was chosen for all further studies.

Effect of initial cadmium (II)and chromium (VI) concentration

The effect of initial cadmium (II) concentration from 5 mg/L-300 mg/L and chromium (VI) concentration from 5 mg/L - 250 mg/L on the removal of cadmium (II) and chromium (VI) from aqueous solutions at biosorbent dose 5 mg/ml and at optimum pH at 30^{0}C temperature was studied. On increasing the initial cadmium (II) concentration, the total cadmium (II) uptake increased appreciably and the total chromium (VI) ions uptake decreased appreciably.

Effect of contact time

Contact time plays an important role in affecting efficiency of biosorption. Contact time is the time needed for biosorption process to achieve equilibrium when no more changes in adsorptive concentration were observed after a certain period of time. The contact time which is required to achieve equilibrium depends on the differences in the characteristics properties of the biosorbents. In order to optimize the contact time for the maximum uptake of cadmium (II), contact time was varied between 5 minutes–180 minutes and for chromium (VI), 10 minutes–180 minutes on the removal of metal ions from aqueous solutions in the concentration of metal ions10 mg/L, biosorbent dose 5mg/ml, at optimum pH and 30^{0}C temperature (Fig. 5). The results obtained from the biosorption capacity of cadmium (II) and chromium (VI) onto the rind of orange (*Citrus sinensis*), (L.) Osbeck showed that the biosorption increases with increase in contact time until it reached equilibrium. The optimum contact time for biosorption of cadmium (II) and chromium (VI) onto the rind of orange (*Citrus sinensis*), (L.)Osbeck was 90 minutes and 150 minutes with maximum removal. The rapid uptake of cadmium (II) is due to the availability of ample active sites for sorption. A further increase in the contact time has a negligible effect on the biosorption capacity. So a contact time of 90 minutes and 150 minutes was fixed for cadmium (II) and chromium (VI) respectively for further experiments.

Adsorption isotherms

The analysis of the adsorption isotherms data by fitting them into different isotherm models is an important step to find the suitable model that can be used for design process. The experimental data was applied to the two-parameter isotherm models: Langmuir, Freundlich, Dubinin-Kaganer-Redushkevich (DKR) and Temkin.

Langmuir adsorption isotherm:

The Langmuir equation, which is valid for monolayer sorption onto a surface of finite number of identical sites, is given by (Langmuir, 1918),

$$q_e = \frac{q_m b C_e}{1+bC_e} \quad (3)$$

Where q_m is the maximum biosorption capacity of biosorbent (mg g^{-1}). b is the Langmuir biosorption constant (L mg^{-1}) related to the affinity between the biosorbent and biosorbate.

Linearized Langmuir isotherm allows the calculation of biosorprtion capacities and Langmuir constants and is represented as,

$$\frac{1}{q_e} = \frac{1}{q_m b C_e} + \frac{1}{q_m} \qquad (4)$$

The linear plots of $1/q_e$ vs $1/c_e$ is shown in Fig. 6 (a). The constants b and q_m are calculated from the slope ($1/q_m \cdot b$) and intercept ($1/q_m$) of the line. The values of q_m, b and regression coefficient (R^2) are listed in Table 1.

Maximum biosorption capacity (q_m) of cadmium (II) and chromium (VI)is found to be83.33mg per gand 10.74mg per g, respectively.

The essential characteristics of the Langmuir isotherm parameters can be used to predict the affinity between the biosorbate and biosorbent using separation factor or dimensionless equilibrium parameters, R_L expressed as in the following equation:

$$R_L = \frac{1}{1+bc_i} \qquad (5)$$

where b is the Langmuir constant and C_i is the maximum initial concentration of metal ions. The value of separation parameters R_L provides important information about the nature of biosorption. The value of R_L indicated the type of Langmuir isotherm to be irreversible ($R_L = 0$), favorable ($0 < R_L < 1$), linear ($R_L = 1$) or unfavorable ($R_L > 1$). The R_L was found to be 0.5181-0.9847 for concentration of 5 mg/L -300 mg/L with respect to cadmium (II) and 0.1503-0.8984 for concentration of 5 mg/L -250 mg/L with respect to chromium (VI). They are in the range of 0-1 which indicates favorable biosorption (Malkoc andNuhoglu, 2005).

Biosorption can also be interpreted in terms of surface area coverage against initial metal ion concentration and separation factor. Langmuir model for surface area of biosorbent surface has been represented in the following equation:

$$bC_i = \frac{\theta}{1-\theta} \qquad (6)$$

where θ is the suface area coverage. The θ was found to be 0.0152-0.4818 for concentration of 5 mg/L - 300 mg/L with respect to cadmium (II) and 0.1015-0.8496 for concentration of 5 mg/L - 250 mg/L with respect to chromium (VI).

Frenudlich adsorption isotherm:

Freundlich equation is represented by (Freundlich, 1906),

$$q = KC_e^{1/n} \qquad (7)$$

where$K$ and n are empirical constants incorporating all parameters affecting the biosorption process such as, biosorption capacity and biosorption intensity respectively.

Linearized Freundlich adsorption isotherm was used to evaluate the sorption data and is represented as,

$$\text{Log } q_e = \log K + \frac{1}{n} \log C_e \qquad (8)$$

Equilibrium data for the biosorption is plotted as log $q_e$vs log C_e, as shown in Fig. 6 (b). The constants n and $K$are calculated from the slope ($1/n$) and intercept (log K) of the line, respectively. The values of K, $1/n$ and regression coefficient (R^2) are listed in Table 1.

The n value indicates the degree of non-linearity between solution concentration and biosorption as followes: if n = 1, then biosorption is linear; if $n < 1$, then biosorption is chemical process; if $n > 1$, then biosorption is a physical process. A relatively slight slope and a small value of 1/ n indicate that, the biosorption is good over entire range of concentration. The n value in Freundlich equation was found to be 2.1505 and 2.7878 with respect to cadmium (II) and chromium (VI).Since n>1, this indicates the physical biosorption for both cadmium (II) and chromium (VI) onto the biosorbent. The higher value of K (10.423) for cadmium (II) and K (3.9801) for chromium (VI),indicates the higher biosorption capacity of the biosorbent.

Dubinin-Kaganer-Radushkevich (DKR) adsorption isotherm:

Linearized Dubinin-Kaganer-Radushkevich (DKR) adsorption isotherm equation is represented as (Dubinin and Radushkevich, 1947),

$$Inq_e = \text{In } q_m - \beta\varepsilon^2 \qquad (9)$$

Where q_m is the maximum biosorption capacity, β is the activity coefficient related to mean biosorption energy and ε is the polanyi potential, which is calculated from the following relation,

$$\varepsilon = RTIn\,(+\frac{\theta}{1-\theta}) \qquad (10)$$

Equilibrium data for the biosorption is plotted as ln$q_e$vsε^2, as shown in Fig. 6 (c). The constants β and q_m are calculated from the slope (β) and intercept (lnq_m) of the line, respectively. The values of adsorption energy E was obtained by the following relationship.

$$E = \frac{1}{\sqrt{-2\beta}} \qquad (11)$$

The values of q_m, β, E and regression coefficient (R^2) are listed in Table 1.

Table 1: Adsorption isotherm constants for biosorption of cadmium (ii) and chromium (vi) byrind of orange (*citrus sinensis*), (l.)Osbeck

Metal	Langmuir constants			Freundlich constants			DKR constants				Temkin constants		
	q_m	b	R^2	K	$1/n$	R^2	q_m	B	E	R^2	A_T	b_T	R^2
Cadmium (II)	83.33	0.0031	0.911	10.423	0.465	0.831	9.1614	-4E-7	1.4526	0.448	4.0212	294.34	0.654
Chromium (VI)	10.74	0.0226	0.998	3.9801	0.3587	0.915	5.3153	-3E-6	0.4082	0.741	2.2009	1364.94	0.890

Table 2: Adsorption kinetic data for biosorption of cadmium (ii) and chromium (vi) byrind of orange (*citrus sinensis*), (l.)Osbeck

Metal	Pseudo-first-order model			Pseudo-second-order model			Elovich model			Intra particle diffusion model		
	q_e	k_1	R^2	q_e	k_2	R^2	a	B	R^2	ki	c	R^2
Cadmium (II)	2.3094	0.047	0.848	1.0729	4.599	0.998	1.3826	6.4935	0.713	0.044	0.584	0.537
Chromium (VI)	1.4605	0.0164	0.901	1.7111	0.0500	0.996	1.5000	4.3233	0.9313	0.062	0.9277	0.873

Table 3 : Thermodynamic parameters of biosorption of cadmium (ii) and chromium (vi) by rind of orange (*citrus sinensis*), (l.)Osbeck

Metal	$-\Delta G^0$ (kJ/mol)				ΔH^0 (KJ/mol)	ΔS^0 (J/mol K)
	293 (Kelvin)	303 (Kelvin)	313 (Kelvin)	323 (Kelvin)		
Cadmium (II)		0.769	0.956	1.680	13.319	46.21
Chromium(VI)	0.211	0.810	0.837	1.185	8.839	30.27

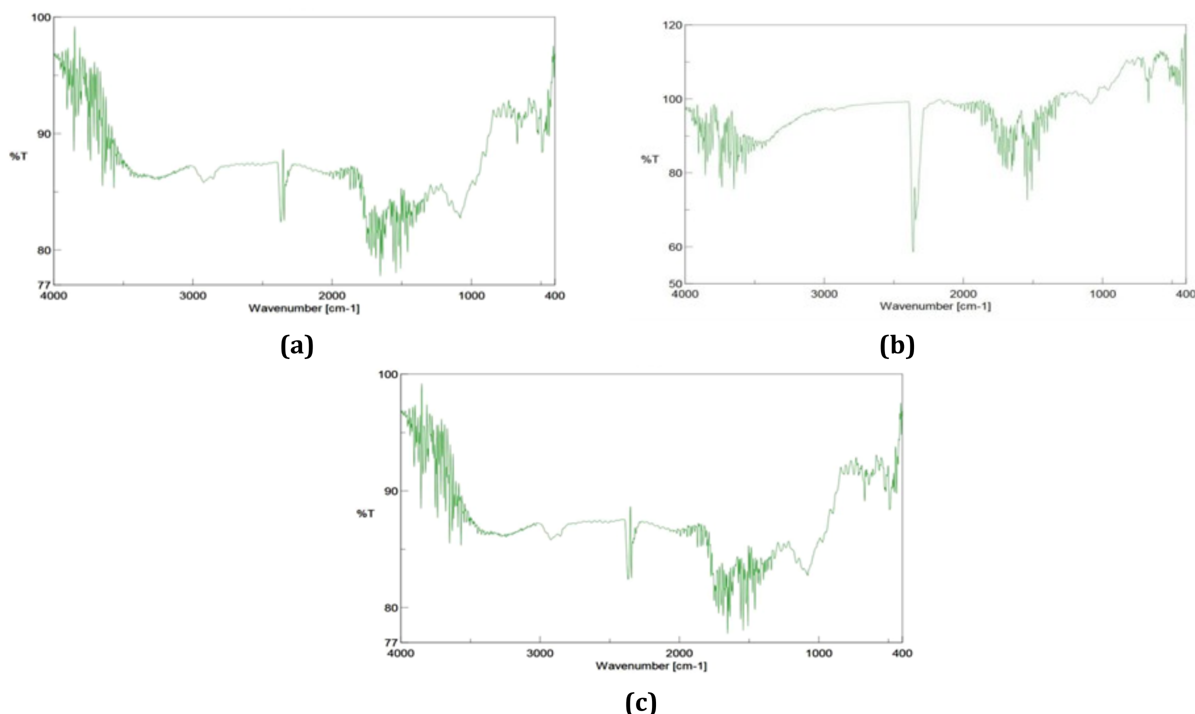


Fig. 1: FTIR spectra (a) biosorbent, rind of orange (*Citrus sinensis*), (L.)Osbeck (b) biosorbent, rind of orange (*Citrus sinensis*), (L.) Osbeck loaded with cadmium (II)(c) biosorbent, rind of orange (*Citrus sinensis*), (L.) Osbeck loaded with chromium (VI)

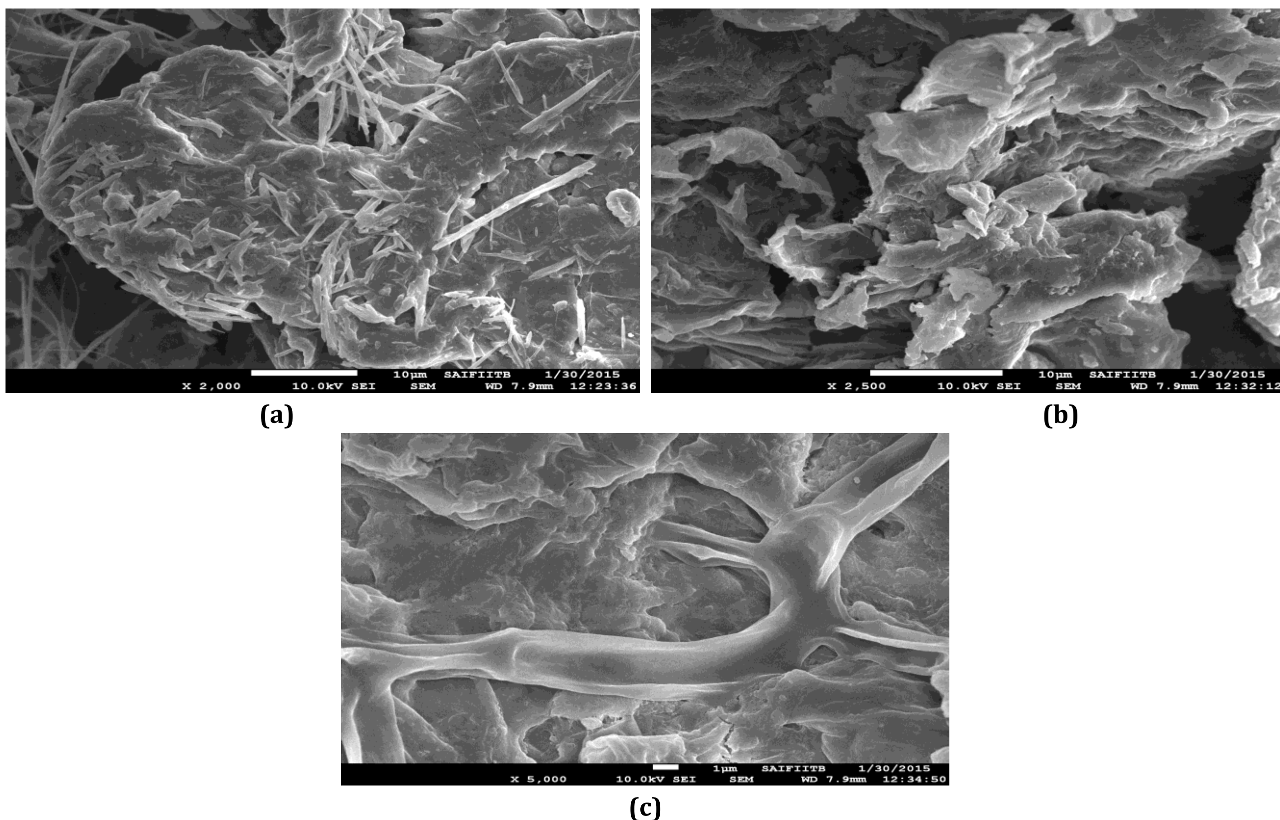


Fig. 2: Scanning Electron Microscope (SEM) analysis (a) biosorbent,rind of orange (*Citrus sinensis*), (L.)Osbeck (b) biosorbent,rind of orange (*Citrus sinensis*), (L.)Osbeck loaded with cadmium (II)(b) biosorbent,rind of orange (*Citrus sinensis*), (L.) Osbeck loaded with chromium (VI)

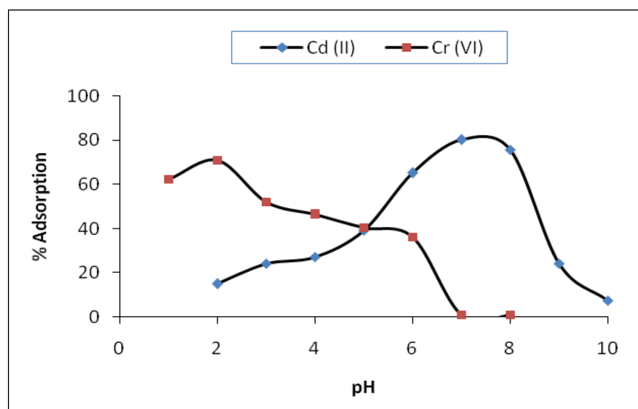


Fig. 3

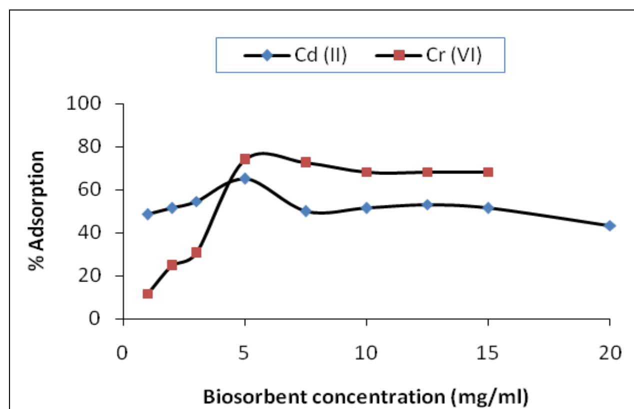


Fig. 4

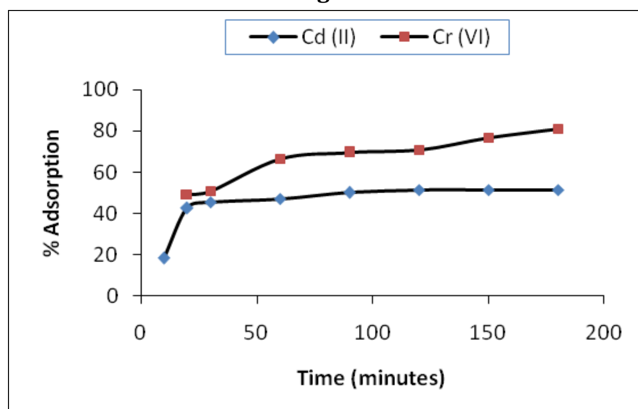


Fig. 3: Effect of pH on biosorption of cadmium (II) and chromium (VI)usingrind of orange (*Citrus sinensis*), (L.)Osbeck

Fig. 4: Effect of biosorbent dose concentration on biosorption of cadmium (II)and chromium (VI)using rind of orange (*Citrus sinensis*), (L.)Osbeck

Fig. 5: Effect of time on biosorption of cadmium (II)and chromium (VI) using rind of orange (*Citrus sinensis*), (L.)Osbeck

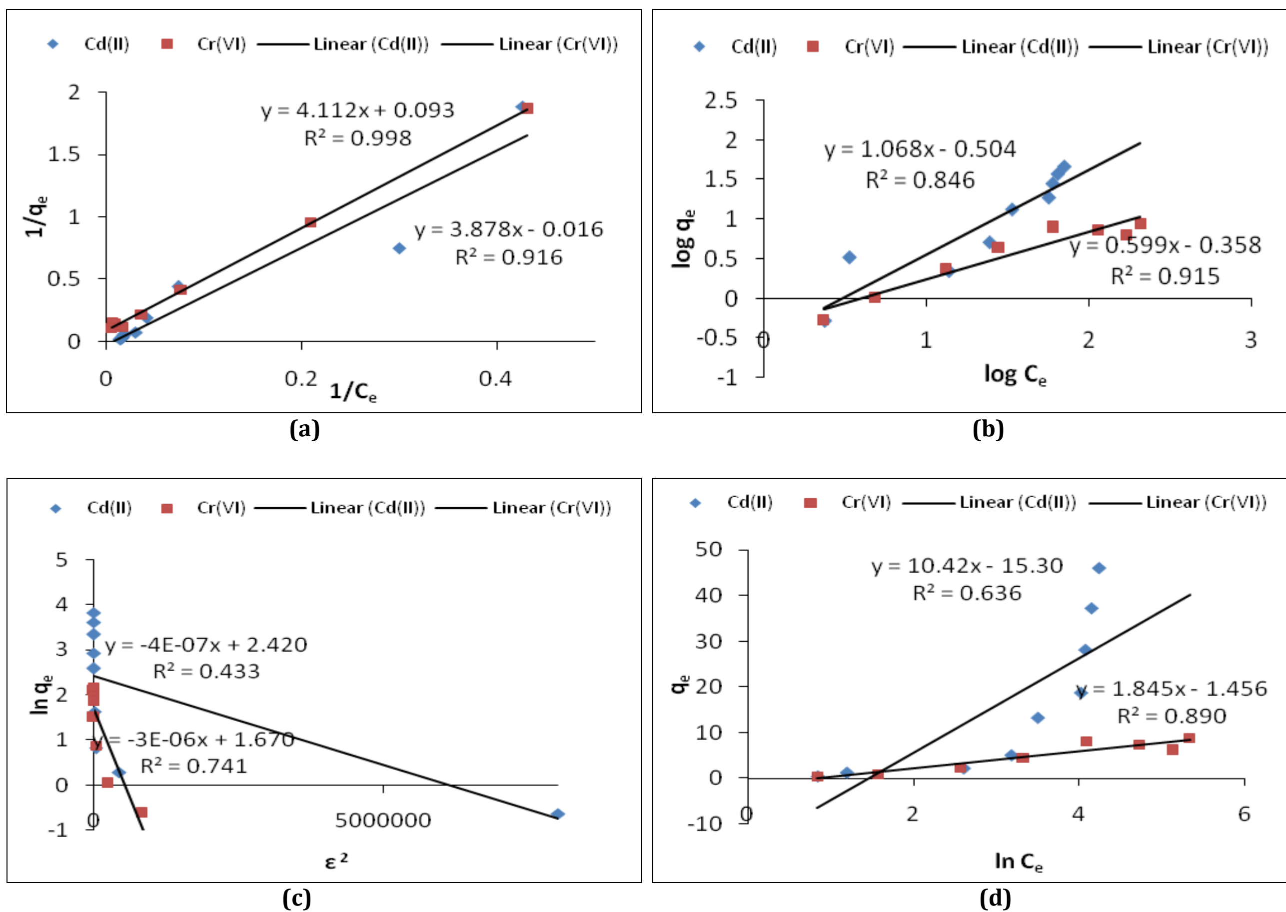


Fig. 6: Adsorption isotherm models (a) Langmuir (b) Freundlich (c) DKR and (d) Temkin, forbiosorption of cadmium (II) and chromium (VI) using rind of orange (*Citrus sinensis*), (L.) Osbeck

The mean free energy gives information about biosorption mechanism, whether it is physical or chemical biosorption. If E value lies between 8 KJ mol^{-1} and 16 KJ mol^{-1}, the biosorption process take place chemically and $E < 8$ KJ mol^{-1}, the biosorption process of the physical in nature (Olivieri and Brittenham, 1997). In the present work, E value (1.118 KJ mol^{-1}) for cadmium (II) and (0.4082 KJ mol^{-1}) for chromium (VI) which is less than 8 KJ mol^{-1}, the biosorption of cadmium (II) and chromium (VI) onto biosorbent, is of physical in nature(Sawalha *et al.*, 2006).

Temkin adsorption isotherm:

Linearized Temkin adsorption isotherm is given by the equation (Temkinand Pyzhev, 1940),

$$q_e = \frac{RT}{b_T}\ln(A_T\, C_e) \qquad (12)$$

Where b_T is the Temkin constant related to heat of biosorption (J/mol) and A_T is the Temkin isotherm constant (L/g).Equilibrium data for the biosorption is plotted as $q_e$vslnC_e, as shown in Fig. 6 (d).The constants $b_T$and A_T are calculated from the slope (RT/b_T) and intercept ($RT/b_T \cdot lnA_T$) of the line, respectively. The values of A_T,b_T and regression coefficient (R^2) are listed in Table 1.

Adsorption kinetics

As aforementioned, a lumped analysis of biosorption rate is sufficient to practical operation from a system design point of view. The commonly employed lumped kinetic models, namely (a) the pseudo-first-order equation (Lagergren, 1898)(b) the pseudo-second-order equation (McKay, 1999)(c) Elovich(Chien and Clayton, 1980) (d) Weber &Morrisintra-particle diffusion (Weber and Morris, 1963) are presented below,

$$\ln(q_e - q_t) = \ln q_e - k_1 t \qquad (13)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad (14)$$

$$q_t = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln t \qquad (15)$$

$$q_t = k_i t^{0.5} + c \qquad (16)$$

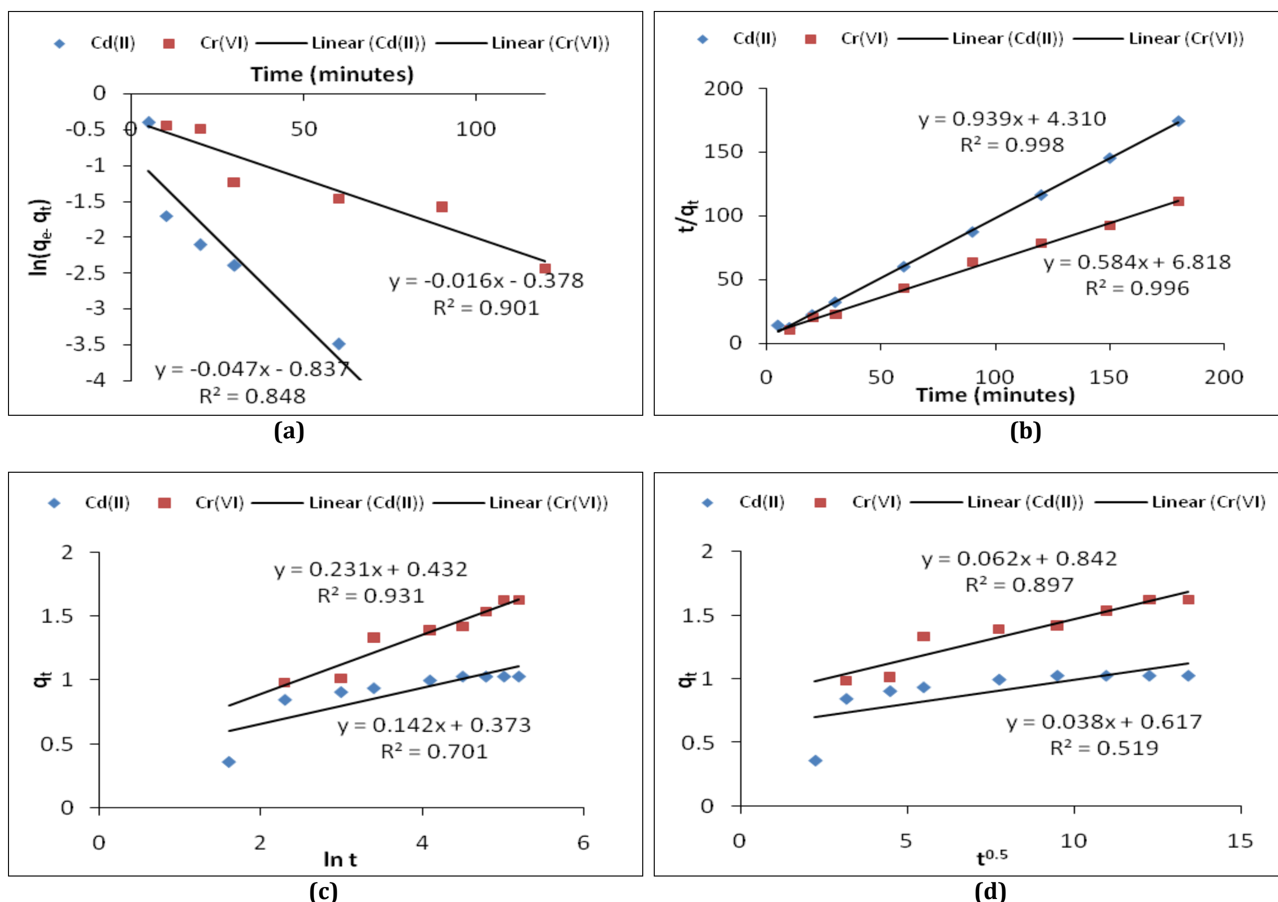


Fig. 7: Adsorption kinetic models **(a)** pseudo-first-order **(b)** pseudo-second-order(c) Elovichand **(d)** Weber and Morris intraparticlerate diffusion, for biosorptionof cadmium (II) and chromium (VI) using rind of orange (*Citrus sinensis*), (L.) Osbeck

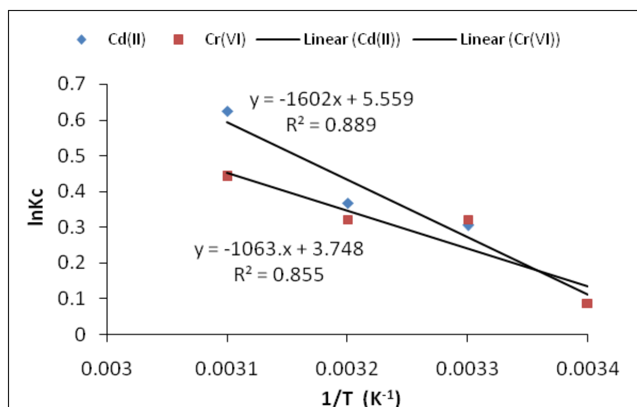


Fig. 8: Determination of thermodynamic parameters for biosorption of cadmium (II) and chromium (VI)using rind of orange (*Citrus sinensis*), (L.) Osbeck

Where q_e (mg g^{-1}) is the solid phase concentration at equilibrium, q_t (mg g^{-1}) is the average solid phase concentration at time t (min), k_1 (min^{-1}) and k_2(g mg^{-1} min^{-1}) are the pseudo-first-order and pseudo-second-order rate constants, respectively. The symbols of α (mg g^{-1} min^{-1}) and β (g mg^{-1}) are Elovich coefficients representing initial biosorption rate and desorption constants, respectively. k_i (mg g^{-1} min$^{-1/2}$) is the intraparticle diffusion rate constant, c is intercept.

If the biosorption follows the pseudo-first-order rate equation, a plot of ln $(q_e\text{-}q_t)$ against time t should be a straight line. Similarly, t/q_t should change lineally with time t if the biosorption process obeys the pseudo-second order rate equation. If the biosorption process obeys Elovich rate equation, a plot of $q_t$against ln$t$ should be a straight line. Also a plot of $q_t$against $t^{0.5}$ changes lineally the biosorption process obeys the weber & Morris intra-particle diffusion rate equation.

Biosorption of metal ions on to biosorbent was monitored at different specific time interval. The metal ions uptake was calculated from the data obtained. From the metal ions uptake was plotted against time to determine a suitable kinetic model, the biosorption data was fitted into pseudo-first-order rate equation, pseudo-second-order rate equation, Elovich equation

and the Weber & Morris intra-particle diffusion rate equation. The pseudo-first-order equation was plotted for ln(q_e-q_t) against t (Fig. 7 (a)). The values of $q_e$and k_1 values were calculated from the slope (k_1) and intercept (lnq_e) of this plot. The values of q_e,$k_1$and regression coefficient (R^2) are listed in Table 2. Kinetic biosorption for pseudo-first-order model occurs chemically and involves valency forces through ion sharing or exchange of electron between the biosorbent and the metal ions biosorbed onto it (Septhum*et al.*, 2007). The pseudo-second-order equation was plotted for t/q_t against t (Fig. 7 (b)). The values of q_e and k_2 are calculated from the slope ($1/q_e$) and intercept ($1/k_2\ q_e^2$) of the plot. The valuesof q_e, $k_2$and regression coefficient (R^2) are listed in Table 2. This suggests that metal ions biosorption occurs in a monolayer fashion and which relies on the assumption that chemisorption or chemical biosorption is the rate-limiting step. Metal ions react chemically with the specific binding sites on the surface of biosorbent. The Elovich equation was plotted for $q_t$against ln$t$ (Fig. 7 (c)). The values of β and α are calculated from the slope ($1/\beta$) and the intercept ($\ln(\alpha\beta)/\beta$) of the plot. The values of β, α andregression coefficient (R^2) are listed in Table 2. The Elovich equation has been used to further explain the pseudo-second-order equation with the assumption that the actual adsorption surface is energetically heterogeneous. Therefore, this could be used to explain that the biosorption surface is energetically heterogeneous (Thomas and Thomas, 1997). The intraparticle diffusion rate equation was plotted for $q_t$against $t^{0.5}$ (Fig. 7 (d)). The value ofk_i and c are calculated from the slope (k_i) and intercept (c) of the plot. The values ofk_i, $c$and regression coefficient (R^2) are listed in Table 2. The intercept of the plot does not pass through the origin, this is indicative of some degree of boundary layer control and intra-particle pore diffusion is not only rate-limiting step(Weber and Morris, 1963). The plot of intra-particle diffusion rate equation showed multilinearity, indicating that three steps take place. The first, sharper portion is attributed to the diffusion of biosorbate through the solution to the external surface of biosorbent or the boundary layer diffusion of solute molecules. The second portion describes ion stage, where intra particle diffusion is a rate limiting. The third portion is attributed to the final equilibrium stage. However the intercept of the line fails to pass through the origin which may attribute to the difference in the rate of mass transfer in the initial and final stages of biosorption (Panday *et al.*, 1986).

Thermodynamic study

The effect of temperature on removal of metal ions from aqueous solutionsin the metal ions concentration 10 mg/L and biosorbentdose 5 mg/ml with optimized pH was studied. Experiments were carried out at different temperatures from 20^0C-70^0C. The samples were allowed to attain equilibrium. Sorption slightly increases from 30^0C-50^0C for cadmium (II) and 20^0C-50^0C for chromium (VI).The equilibrium constant(Catena and Bright, 1989)at various temperatures and thermodynamic parameters of adsorption can be evaluated from the following equations,

$$K_c = \frac{C_{Ae}}{C_e} \quad (17)$$

$$\Delta G^0 = -RT\ \ln K_c \quad (18)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (19)$$

$$lnK_c = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \quad (20)$$

Where K_c is the equilibrium constant, C_e is the equilibrium concentration of metal ions in solution (mg/L) and C_{Ae} is the metal ionsconcentration biosorbed on the biosorbent per liter of solution at equilibrium (mg/L). ΔG^0, $\Delta H^0$and $\Delta S^0$are changes in standard, Gibbs free energy (kJ/mol), enthalpy (kJ/mol) and entropy (J/mol K), respectively. R is the gas constant (8.314 J/mol K),T is the temperature (Kelvin). The values of ΔH^0 and ΔS^0 were determined from the slope ($\Delta H^0/R$) and the intercept ($\Delta S^0/R$) from the plot of lnKc versus $1/T$(Fig. 8.). The values of standard Gibbs free energy change (ΔG^0), standard enthalpy change (ΔH^0) and the standard entropy change (ΔS^0) calculated in this work were presented in Table 3. The equilibrium constant (Kc) increases with increase in temperature, which may be attributed to the increase in the pore size and enhanced rate of intraparticle diffusion.The standard Gibbs free energy (ΔG^0) is small and negative and indicates the spontaneous nature of the biosorption. The values of Gibbs free energy (ΔG^0) were found to decreases as the temperature increases, indicating more driving force and hence resulting in higher biosorption capacity. The value of standard enthalpy change (ΔH^0) was positive, indicating the endothermic nature of the biosorption of metal ions onto the biosorbent. The positive values of standard entropy change (ΔS^0) shows an affinity of biosorbent and the increasing randomness at the solid solution interface during the biosorption process.

CONCLUSIONS

The present investigation revealed that the rind of orange (*Citrus sinensis*), (L.) Osbeck can be an inexpensive, excellent biosorbent for the removal of cadmium (II) and chromium (VI) from aqueous solutions.FTIR analysis of biosorbent confirmed that hydroxyl, carbonyl and carboxyl group, so that the cell wall surface of the biosorbent that may interact with the zinc (II) and chromium (VI). The SEM represents a porous structure with large surface area. The optimal parameters such as solution pH, biosorbent dose, initial metal ions concentration, contact time and temperature determined in the experiment were effective in determining the efficiency of cadmium (II) and chromium (VI) ontothe rind of orange (*Citrus sinensis*), (L.) Osbeck.Langmuir isotherm model provided a better fit with the experimental datafor both cadmium (II) and chromium (VI). The maximum biosorption capacity of cadmium (II) and chromium (VI) which was determined from Langmuir isotherm was found to be 83.33 mg $g^{1}$and 10.74mg g^{-1}respectively.Results clearly indicates that the pseudo-second-order kinetic model was found to be correlate the experimental data strongest for both cadmium (II) and chromium (VI). The thermodynamic study confirmed thatreaction of biosorptionof cadmium (II) and chromium (VI) onto the rind of orange (*Citrus sinensis*), (L.) Osbeckis spontaneous, endothermicand increasing randomness of the solid solution interfaces. From these observations it can be concluded that the rind of orange (*Citrus sinensis*), (L.) Osbeck has considerable biosorption capacity, available in abundant, non-hazardous agro material could be used as an effective indigenous material for treatment of wastewater stream containing cadmium (II) and chromium (VI).

REFERENCES

Ahluwalia SS and Goyal D (2007) Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresour. Technol.,* 98:2243-2257.

Bhalerao SA (2011) Biosorption: an eco-friendly, cost effective technology for removal of heavy metals an overview. *Bionano frontier, special issue,* 219-225.

Catena GC and Bright FV (1989) Thermodynamic study on the effect of cyclodixtrin inclusion with anilinonaphthalenesulphonates. *Anal. Chem., 61:905-909.*

Chien SH and Clayton WR (1980) Application of Elovich equation to the kinetics of phosphate release and sorption in soils, *Soil Sci. Soc. Am. J.* 44:265-268.

CiminoG and Caristi C (1990)Acute toxicity of heavy metals to aerobic digestion of waste cheese whey. *Biol. Waste,* 33:201-210.

Dubinin MM and Radushkevich LV (1947) Equation of the characteristic curve of activated charcoal, *Proc. Academy of Sci. Phy. Chem. Section, U.S.S.R.,* 55:331-333.

Freundlich HMF (1906) Uber die adsorption in losungen, *ZeitschriftfuePhysikalischeChemie (Leipzig)*, A57:385-470.

Karthikeyan S, Balasubramanian R and Iyer CSP (2007) Evaluation of marine algae Uivafasciata and Sargassum sp. for biosorption of Cu (II) from aqueous solutions. *Bioresour. Technol.,* 98 (2): 452-455.

Kratochvil D and Volesky (1998) Advances in the biosorption of heavy metals. *Trends inBiotechnology,* 16(7):291-300.

Lagergren S (1898) About the theory of so-called adsorption of soluble substances, *KungligaSvenskaVetenskapsakademiensHandlingar.*24:1-39.

Langmuir I, (1918) The adsorption of gases on plane surface of glass, mica and platinum, *J. Am. Chem. Soc.,* 40:1361-1403.

Maind SD, Rathod SV, Gajbhiye S, Hile VK and Bhalerao SA (2012)Biosorption of copper (II) ions from aqueous solutions by moss (*Semibarbulaorianttalis* (web.) Wijk. & Marg.),*Int. J. Environmental Sciences.* 1(4):402-414.

Maind SD, RathodSV and Bhalerao SA (2013) Batch adsorption studies on removal of Fe (II) ions from aqueous solutions by corn cobs (*Zea mays* Linn.), *Int. J. Chem.,* 2:136-148.

Maind SD,RathodSV and Bhalerao SA (2012) Biosorption of copper (II) from aqueous solutions by corn cobs. *J. Biological & Physical Sciences, special issue.* 1:111-115.

MaindSD and Bhalerao, SA (2013) Removal of Fe(II) ions from aqueous solutions using moss (*Semibarbulaorientalis* (web.) Wijk. & Marg.) in a batch system, *Int. J. Biotech. Chem. & Environ. Eng.* 2(2):26-35.

Malkoc E and Nuhoglu YJ (2005) Investigation of Nickel (II) removal from aqueous solutions using tea factory waste, *J. Hazard. Mater.* B127:120-128.

McKay G, Ho YS and Ng JCY (1999)Biosorption of copper from waste waters: A review, *Sep. Purif. Methi.* 28: 87-125.

Mungasavalli,DP, ViraraghavanT and JINYee-Chung (2007) Biosorption of chromium from aqueous solutions by pretreated *Aspergillusniger*: Batch and column studies. *Colloids and Surfaces A:Physicochemical Engineering Aspects,*301(1-3): 214-223.

Naiya TK, Das SK and Bhattacharya AK (2009) Adsorption of Cd (II) &Pb (II) from aqueous solution on activated alumina. *J. Coll. Inter. Sci.,* 21: 434-451.

Olivieri NF and Brittenham GM (1997) Iron-chelating therapy and the treatment of thalassemia, *Blood,* 89:739-761.

Panday KK, Prasad G and Singh VN (1986) Mixed adsorbents for Cu(II) removal from aqueous solutions. Environ. Technol. Lett., 50 (7): 547-554.

Patterson JW (1985) Industrial Wastewater Treatment Technology, *Butterworth Publication, Stoneham.*

Perez-Marin AB, Zapata VM, Ortuno JF, Aguilarn M, SaezJ and Llorens, M (2007) Removal of cadmium by aqueous solutions by adsorption onto orange waste. *J. Hazard. Mater.,*139:22-131.

Pino GH,Souza de Messquita LM, ToremML and Pinto GA (2006) Biosorption of cadmium by green coconut shell powder. *J. Minerals Eng.* 19:380-387.

Rao PS, Mise RS and Munjunatha GS (1992) Kinetic studies on adsorption of chromium by coconut shell carbons from synthetic effluents. *J. Environ. Sci. Health A,* 27: 2227-2241.

Sawalha MF, Peralta-Videa JR, Romero-Gonzalez J and Gardea-Torresdey JL (2006)Biosorption of Cd(II), Cr(III) and Cr (VI) by saltbush (Atriplexcanescens) biomass : Thermodynamic and isotherm studies, *J. Colloid Interface Sci.,* 300:100-104.

Septhum C, Rattanaphani S, Bremner JB and Rattanaphani V (2007) An adsorption of Al (III) ions onto chitosan. *J. Hazardous Materials.* 148:185-191.

Sharma YC (2008) Thermodynamics of removal of cadmium by adsorption on indigenous clay. *Chem. Eng. J.,* 145:64-68.

Temkin MJ and Pyzhev V (1940) Kinetics of ammonia synthesis on promoted iron catalysts, *ActaPhysiochim. Urrs,* 12:217-222.

Thomas JM and Thomas WJ (1997) Principle and Practice of heterogeneous catalysis, *weinheim, VCH.*

Volesky B (2013) Sorption and biosorption. Montreal-St. Lambert, *Quebec, Canada, BV Sorbex Inc.* 316.

Weber WJ and Morris JC (1963) Kinetics of adsorption on carbon solution. *J. SanitEng. Div. Am. Soc. Civ. Engg.* 89: 31-59.

WHO (3rd ed.), (2008) Guidelines for Drinking Water Quality: Recommendations, *World Health Organization Geneva, Vol 1.*

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Effect of temperature on the antimicrobial activity of lime juice-honey syrup on certain bacterial isolate

Unegbu Chika C[1*], Njoku SC[1] and Ajah Obinna[2]

[1]Department of chemistry/biochemistry, Federal Polytechnic Nekede, Owerri, Imo State, Ngeria
[2]Department of Pharmaceutical Technology, Federal Polytechnic Nekede, Owerri, Imo State, Ngeria.
*Corresponding author Email: buffaogb@yahoo.com

Manuscript details:

Received: 15.02.2016
Accepted: 24.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Unegbu Chika C, Njoku SC and Ajah Obinna (2016) Effect of temperature on the antimicrobial activity of lime juice-honey syrup on certain bacterial isolate. *International J. of Life Sciences*, 4(1): 57-61.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

Traditionally in Nigeria, lime juice (*Citrus aurantifolia*) is added to honey and often used as a cough medicine. The present study is aimed at determining the effect of the temperature on the mixture of lime juice and honey and its antimicrobial activity on selected bacteria isolates. The lime juice is squeezed out of the fruit and then mixed with honey in the ratio of 1:1, 2:1 and 1:2 respectively, the mixture are then heated for 15minutes at various temperature (40, 60, 80 and 100°C). The result shows that the mixture still has antibacterial activity on heating. The heating temperature of 40°C, 60°C and 80°C at the ratio of 1:1 and 2:1 showed zones of inhibition when compared with the unheated but the heating temperature of 100°C has no zone of inhibition on the isolates. This indicates that heating can affect the antibacterial activity of the mixture and the evidence suggests that the lime is majorly responsible for the antibacterial activity.

Keywords: Antibacterial, bacteria isolate temperature, lime juice and honey

INTRODUCTION

Several medicines or substances are used to suppress or relieve coughing. Anti-tussives agents are said to work by reducing the cough reflex. It is regarded as an antimicrobial. An antimicrobial is a substance that kills or inhibits the growth or action of microbes such as as bacteria, fungi, protozoa or viruses. Various types of antimicrobial agents includes antibiotics which are generally used against bacteria are used specifically for treating viral infection, antifungal are used to treat fungal infections, some of the side effects of these antimicrobial agents can be life threatening, if the drugs are not used properly (Anderson eta l., 2008).

According to WHO, honey is recommended for coughs in developing countries where is limited acces to medicine. So although the evidences isn't strong, it suggested there may be an effect in acute cough in children

Furthermore, according to the WHO, any plant which contains substance that can be used for therapeutic purpose or which are precursor of chemo-pharmaceuticals. Semi synthetic new drugs are referred as "medicinal plants". Therefore medicinal plants and natural substances would be the best source to obtain a variety of drugs as the phytochemicals and natural substances would be the best source to obtained a variety of drugs as the phytochemicals and components are more specific (Cohen *et al*., 2000).

Honey mixed with lime to form syrup had been used over the years as a redemy for cough in adults and children but mostly in children (De-Castillo *et al*., 2000). This is because the constituents (lime and honey) are safer than over –counter medications. The main objective of this study is the evaluate the effect of temparture on the antibacterial effect of mixed lime juice and honey on certain bacteria isolate and to know among the mixture which one shows the most antibacterial activity (Froloy and Peressadin, 2006).

MATERIALS AND METHODS

Sample Collection

Fresh lime fruits were purchased from Umunam Imerienwe in Ngor-Okpala L.G.A., Imo State, Fresh and raw natural honey was purchased from a local farmer at Itu Ezinihitte Mbaise in Imo State.

Sources of test organisms

The pure clinical isolates of *Staphylococcus eureus, Staphylococcus pyogenes* and *Staphylococcus pneumonia* were collected form fernme medical laboratory Rosana Medical center, Umudubia. Nekede, Imo State.

Extraction of lime juice

The lime fruits were properly washed and sliced into two halves each. Each fruit was gently squeezed and the juice was collected into a sterile beaker. Then the resulting lime juice was filtered through a sterile cheese cloth. After filtration, the filtrate was dispensed into a sterile transparent bottle with cap and the residual pulp and seeds were discarded.

Preparation of Sample

2.5ml of lime juice and 2.5ml of honey was dispended into sterile boiling flasks to form the ratio 1: 1. 2.5ml lime juice and 5ml of honey was dispensed into boiling tubes to form the ratio 1: 2 and 5ml lime juice and 2.5ml of honey was also dispensed into boiling tube to form the ratio 2: 1 of lime-juice honey mixture. The mixtures of each respective ratios were heated in a water bath at 40°C, 60°C, 80C and 100°C for 15minutes. The control mixtures were unheated.

Antimicrobial activity of the mixture

The antimicrobial activity was carried out using the well diffusion method (Owhe-Ureghe *et al*., 2010). The Mueller-Hinton agar was prepared according to the manufacturer's instruction and allowed to cool to 45°C. Then a suspension of each pathogen was made. The medium was dispensed into 3 conical flasks and 1ml of each pathogen suspension was inoculated into the 3 molten agar medium respectively. Each molten agar medium was poured into 15 already autoclaved petri-dishes making a sum total of 45 petri-dishes. The media was allowed to solidify. The glass rod was dipped in alcohol and flamed to sterilize it, and then it was used to bore 6mm diameter wells into all the media. For the plates labeled with *Streptococcus pyogenes*; the 15 petri-dishes were inoculated as; the first 5 plates were inoculated by pouring the rnixture containing 1: 1 ratio of the syrup in the plates as; Plate 1 was inoculated with the syrup heated at 40°C. Plate 2 was inoculated with the syrup heated at 60°C. Plate 3 was inoculated with the syrup heated at 80°C. Plate 4 was inoculated with the syrup heated at 100°C and Plate 5 was inoculated with the unheated syrup. The plates labeled with *Streptococcus pneumonia* and *Staphylococcus aureus* were also inoculated in the same way as stated above, the same pattern of inoculation was repeated with other ratios 1: 2 and 2: 1 using the same organisms. After inoculation, all the plates were incubated at 37°C for 24hours. Then the diameter of each zone of inhibition was measured.

RESULTS

Table 1 represents the results that emanated from the antimicrobial activity of the lime-juice and honey syrup mixture in the ratio 1: 1 (2.5ml and 2.5ml) at various temperatures (40°C, 60°C, 80°C, and 100°C).

Table 2 contains the results gotten from the antimicrobial activity of the mixture in the ratio 1:2 (2.5ml + 5.0ml) respectively at various temperatures as indicated.

Table 3 shows the results from the antimicrobial activity of the lime-juice and honey mixture in the ratio2: 1 (5.0ml + 2.5ml) respectively,

The diameter of the inhibition zones recorded in table 1indicate that the lime juice-honey syrup mixture heated at temperatures 40°C, 60°C and 8O°C was very effective in inhibiting the growth of *Streptococcus pneumonia* with the highest zone of 17mm recorded at 40°C.

Table 2 showed no significant increase in the antibacterial activity of the syrup even when the ratio of honey was increased. Nevertheless, at 60°C, the syrup had a significant effect on *Streptococcus pyogenes*.

Table 3 showed a marked increase in activity of the syrup from that of table 2. At 40°C and 60°C, the syrup had notable effects on the organisms.

Table 1: Effect of temperature on the antimicrobial activity of mixed juice and honey syrup ratio 1: 1

Temperature (^{0}C)	**Organism**	**Zone diameter (mm)**
40	*Streptococcus pyogenes*	10
	Streptococcus pneumonia	17
	Staphylococcus aureus	10
60	*Streptococcus pyogenes*	13
	Streptococcus pneumonia	15
	Staphylococcus aureus	12
80	*Streptococcus pyogenes*	12
	Streptococcus pneumonia	16
	Staphylococcus aureus	12
100	*Streptococcus pyogenes*	2
	Streptococcus pneumonia	-
	Staphylococcus aureus	1
Unheated	*Streptococcus pyogenes*	9
	Streptococcus pneumonia	9
	Staphylococcus aureus	10

Table 2: effect of temperature on the antimicrobial activity of mied lime juice and honey syrup ratio 1: 2

Temperature (^{0}C)	Organism	Zone diameter (mm)
40	*Streptococcus pyogenes*	10
	Streptococcus pneumonia	10
	Staphylococcus aureus	10
60	*Streptococcus pyogenes*	14
	Streptococcus pneumonia	10
	Staphylococcus aureus	11
80	*Streptococcus pyogenes*	7
	Streptococcus pneumonia	9
	Staphylococcus aureus	7
100	*Streptococcus pyogenes*	-
	Streptococcus pneumonia	-
	Staphylococcus aureus	-
Unheated	*Streptococcus pyogenes*	9
	Streptococcus pneumonia	10
	Staphylococcus aureus	10

Table 3: effect of temperature on the antimicrobial activity of mixed juice and honey ratio 2: 1

Temperature (^{0}C)	Organism	Zone diameter (mm)
40	*Streptococcus pyogenes*	14
	Streptococcus pneumonia	13
	Staphylococcus aureus	13
60	*Streptococcus pyogenes*	12
	Streptococcus pneumonia	13
	Staphylococcus aureus	9
80	*Streptococcus pyogenes*	11
	Streptococcus pneumonia	12
	Staphylococcus aureus	10
100	*Streptococcus pyogenes*	-
	Streptococcus pneumonia	-
	Staphylococcus aureus	-
Unheated	*Streptococcus pyogenes*	12
	Streptococcus pneumonia	11
	Staphylococcus aureus	13

Comparing the diameter of the zones recorded in the various tables, it could be observed that with regards to the ratio, the 1: 1 and 2: 1 ratios were more effective than the 1: 2 ratio of the syrup at various temperatures of 40°C, 600C and 8O°C, suggesting that the lime juice could be majorly responsible for the antimicrobial activity of the syrup. The most effective heating temperature for processing the syrup ranges between 40-80°C. This assertion is based on the larger zones of inhibitions recorded at these temperatures. There was also notable inhibition zones resulting from the activity of the unheated syrup at various ratios, which were slightly lower than those recorded at temperatures 40°C, The antimicrobial activity of the syrup in various ratios at100°C showed no zone inhibition zones.

DISCUSSION

The addition of honey in the treatment of cough and sore-throat traditionally might be intended to reduce the Sour taste of lime and to provide nutrients for cough sufferers. In addition, honey has a water activity (aw) of 0.6 leading to the inability of the growth of most micro-organisms (Boukraa, 2010). It also contains antioxidant compounds such as ascorbic acid, chrysin, catalase etc. it also has low pH between 3.2-4.5 which is able to prevent the growth of most bacterial. These are possible reasons for the addition of honey in treated sore throat and cough. The antimicrobial activity of lime has already been established against various micro-organisms. The process of heating the mixture of lime juice and honey before it is used in the traditional treatment of sore-throat and cough might be intended to sterilize it by killing microbial contaminants (Stephen, 2000). It could also be to reduce the thickness of the honey as it was observed in this study that on the application of heat, the honey in the mixture became less sticky and therefore more drinkable and easier to swallow.

The results suggest that the antibacterial activity of a mixture of lime juice and honey, which is indicated by the zones of inhibition, was influenced by temperature. In other words, the antibacterial activity was unstable on high heating. This is presumably due to the fact that temperatures within the ranges 40-80°C but not as high as 100°C in 15munites can stimulate or accelerate the reaction of the active components in order to inhibit the growth of bacteria.

CONCLUSION AND RECOMMENDATION

The results obtained from this study to a large extent revealed a significant antimicrobial activity of lime juice and honey mixture on the bacteria, *Streptococcus pyogenes*, *Streptococcus pneumonia* and *Staphylococcus aureus* at various temperature ranging from 40°C to

80°C within the time intervals of 15minutes on each exposure. It is therefore recommended that further research studies be carried out on this syrup in the bid to develop new biotechnological or pharmaceutical strategies to making it a more effective, and reliable sore throat and cough remedy, not only to locals but also in modern medicine as the effect of temperature on its antimicrobial efficiency is now clearly ascertained.

REFERENCES

Anderson RC, Tomita G, Wlaker S, Merkel R, Pinchak WE and Min BR (2008) Comparative antimicrobial activity of tannin extracts from perennial plants on mastitis pathogens, scientific. Research and Essay, 3(2),66-73.

Boukraa IE (2010) Honey in traditional and modern medicine, CRe press Taylor and Francis group.

Cohen HA, Rozen J, Kristal H, Laks Y, Berkoritch M, Uziel Y, Kozer E, Pomeranz A, Efrat H, (2000) Effect of honey on nocturnal cough and sleep quality: A doubleblind randomized placebo controlled study. Pediatrics, 1-8.

De Castillo MC, De Alloric C, De Guteieirez BC (2000) Bactericidal activity of lemon juice and lime against vibrio cholera biological pharmacy. 23: 1235-1238.

Frolov VM and Peressadin NA (2006) Honey against *influenza* and sore throat, pcelovodstvo10 (529), 52-53.

Owhe-Ureghe UB, Ehwarieme DA and Eboh DO (2010) Antibacterial *activity* of garlic and lime on isolates of extracted carious teeth, *African journal of Biotechnology,* 9(21) 3163-66

Stephen HJ (2004) Evaluation of honey impregnated tulle dressing in primary care. *Britain Journal community Nurs; wound care supplement* 2:521-527.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Effects of sunlight exposure vs. commercial vitamin D supplementation on the bone health of vitamin D deficient rats

Mahmoud Mustafa Ali Abulmeaty MD[1,2]

[1]King Saud University, Department of Community health sciences, Collage of Applied Medical sciences, Riyadh, KSA.
[2]Zagazig University, Obesity research and management unit, Department of Medical Physiology, Faculty of Medicine, Zagazig, Egypt.
Email: Dr.abulmeaty@gmail.com | mabulmeaty@ksu.edu.sa

Manuscript details:

Received: 08.03.2016
Accepted: 21.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Mahmoud Mustafa Ali Abulmeaty MD (2016) Effects of sunlight exposure vs. commercial vitamin D supplementation on the bone health of vitamin D deficient rats. *International J. of Life Sciences*, 4(1): 63-70.

Acknowledgements
The author would like to extend his sincere appreciation to the Deanship of Scientific Research at King Saudi University, KSA for its funding thisresearch group NO(RGP- 193).

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

This study compared sun exposure with commercial vitamin D3 supplementation in restoring bone health in a vitamin D deficient rat model. 35 healthy weaned male albino rats were divided into two groups. Vitamin D deficient rats (n=21) were subdivided into: an Sd group (n=7) that was directly exposed to sun-light, a Tt group (n=7) that was treated with commercial vitamin D3 with oil, and a Ct group (n=7) that was treated with canola oil without vitamin D. Normal diet fed rats were subdivided into: a Cs group (n=7) that was exposed to sunlight and fed a normal vitamin D chow and a control (C) group(n=7). Plasma 25 hydroxy vitamin D3, parathyroid hormone, calcium, phosphorus levels and the enzymatic activity of alkaline phosphatase were estimated and femur bones were used to prepare histopathological sections. The sun-exposed groups showed a significant reduction in levels of parathyroid hormone (67.69±13.18 vs 86.05±9.67 pg/mL, $P < 0.05$) in addition to an improvement of osteoid area and a reduction of trabecular separation in the bone sections. In a conclusion, sun-exposure have a more positive effect on bone structure and hormones that control bone mass rather than normalization of vitamin D.

Key words: Sunlight exposure, vitamin D supplementation, vitamin D deficiency, bone histology.

INTRODUCTION

Vitamin D deficiency is a highly prevalent metabolic condition worldwide (Holick and Chen 2008).Despite the sunny weather in the Middle East, spanning latitudes from 12N to 42N that allow for vitamin D synthesis year round, this region reports individuals with some of the lowest levels of vitamin D and the highest rates of hypovitaminosis D worldwide (Fuleihan, 2009). For instance, in Saudi Arabia the prevalence of vitamin

D deficiency was estimated to be 29% of studied population, which also showed a 22.7% relative insufficiency state (Alsuwadia *et al.,* 2013). This major metabolic problem affects populations at all developmental stages, including pregnant women, neonates, infants, children and adolescents, adults, and the elderly. In addition, maternal vitamin D levels strongly correlate with neonatal levels (El Rifai *et al.,* 2014). Vitamin D deficiency in children causes growth retardation, which classically manifest with symptoms of rickets (Huldschinsky, 1919). In adults, vitamin D deficiency will precipitate and exacerbate osteopenia and osteoporosis and increase the risk of pathological bone fractures (Larsen *et al.,* 2004; Bakhtiyarova *et al.,* 2006). Vitamin D deficiency not only causes metabolic bone disease among children and adults but also may increase the risk of many common chronic diseases such as diabetes, obesity, autoimmune diseases, cancers and cardiovascular disease (Holick, 2004).

Solar UV-B (wavelengths of 290-315 nm) irradiation of the skin via direct sunlight exposure is the primary source of vitamin D for most people (Holick, 2002; Holick and Chen, 2008). Dietary sources of vitamin D are limited. They include oily fish such as salmon, mackerel, and sardines; some fish oils such as cod liver oil; and egg yolks. In addition, some foods including milk and some cereals, orange juice, some yogurts, and margarine are fortified in many countries, by variable amount and forms of vitamin D (Holick *et al.,* 1992; Tangpricha *et al.,* 2003).

Despite vitamin D fortification and dietary counseling efforts that emphasize ingestion of vitamin D from dietary sources, vitamin D deficiency occurs in a pandemic fashion. This suggested the value and roles of sun exposure as a preventive and curative remedy for vitamin D deficiency. The wearing of traditional clothes, the deliberate avoidance of the sun and an indoor life-style may in part explain the high prevalence of vitamin D deficiency (Alsuwadia *et al.,* 2013; Green *et al.,* 2015). Additionally, vitamin D resistance resulting from calcium deficiencies has also been described in some populations in year-round sunny areas, which can lead to hypovitaminosis D (Green *et al.,* 2015).

Rat models of hypovitaminosis D are frequently used in vitamin D research. Dietary deprivation of vitamin D and keeping animals away from sunlight are used as traditional models (Lester *et al.,* 1982; Stavenuiter *et al.,* 2015). The variable roles of sunlight exposure and dietary vitamin D supplementation on bone structure and health are best studied in animal models as they preclude any human related behavioral and cultural factors that may affect vitamin D and calcium metabolism. Taking these factors into consideration, this study was carried out to demonstrate and compare the effects of sun light exposure vs vitamin D_3 supplementation on bone histology and plasma levels of some bone-related hormones and minerals, such as 25 hydroxy vitamin D_3, Parathyroid hormone, calcium, phosphorus and alkaline phosphatase in a rat model of hypovitaminosis D.

MATERIALS AND METHODS

Animals:

A total of 35 healthy male albino rats were used. Shortly after weaning, 3- to 4-week old rats (body weight 78.70±10.2 g) were usedin accordance with an experimental protocol approved by the ethics committee of College of Applied Medical Sciences, King Saud University. All of the rats were bredin light-temperature-controlled animal housing (12-h light, 12-h dark cycle, temperature approximatly 25 C^o, respectively). The rats were divided primarily into two groups: those in group I (n=14) were fed on a normal balanced growth diet (AIN-93G, Bio-Serve, USA) (Reeves *et al.,* 1993) in a normally lit room. The remaining rats in group II (n=21) were fed a customized; vitamin D deficient, normal calcium and phosphorus diet (Custom AIN-93G, Bio-Serve, USA)(table 1)(Bio-Serv 2015) for 6 weeks in covered cages to limit direct exposure to florescent light.

After 6 weeks, the rats in group I were subdivided into two subgroups; the Ss group was exposed to sunlight and fed same diet of group I (vitamin D 1000 IU/kg) for 10 days and the C group, which received no intervention but continued feeding on the group I diet. In another set of experiments, rats in group II were subdivided into 3 subgroups. The Sd group (n=7) was directly exposed to sun-light. The Tt group (n=7) was treated with commercial vitamin D_3 with canola oil as 3 ml of a 27 ug/ml vitamin D_3 solution (Fleet *et al.,* 2008)(MUP co, Egypt) administered together with an equal amount of pure canola oil divided into 3 doses over the treatment period. The Ct group (n=7) was treated with 3 ml of canola oil without vitamin D for 10 days. One rat from Ct group died during the study.

Table 1: General futures of composition of rat's diets (16)

Item	Group I diet	Group II diet
Protein	18.1 %	18.1 %
Fat	7.1 %	7.1 %
Carbohydrate	59.3 %	59.3 %
Total energy	3.74 kcal/gm	3.74 kcal/gm
Fiber	4.8 %	4.8 %
Ash	2.2 %	2.2 %
Calcium	5.1 gm/kg	5.1 gm/kg
Phosphorus	2.8 gm/kg	2.8 gm/kg
Vitamin D3	1000 IU/kg	0-50 IU/kg

Sun light exposure:

The Sd and Ss subgroups were exposed to direct sunrays from 1:30 to 2 pm during May 2015 in Riyadh Saudi Arabia (latitude 24°N). No forms of sun-screen or protection were used to protect the sun-exposed groups; rather, they were left in an open field in separate cages to reduce thermal exhaustion and sweating.

Blood sampling:

Blood samples (0.5 ml/rat) were obtained from tail veins before the treatment period. At the end of the study, after overnight fasting, blood (5-6 ml/rat) was collected via cardiac puncture from the rats while under deep anesthesia (3-5% isoflurane in a vaporizer chamber), followed immediately by cervical dislocation as an appropriate and humane method of euthanasia. Blood samples were collected in green toped, heparinized tubes (Greiner Bio-One Germany) and then centrifuged for 15 minutes at approximately 500 rpm. The separated plasma supernatant was stored at -80°C untill the time of measurement. Repeated freezing and thawing were avoided.

Biochemical analysis:

1. Measuring plasma 25 hydroxy vitamin D and parathyroid hormone (PTH) levels: ELISA kits (Mybiosource USA) were used according to the manufacturer's protocols (Shuai *et al.,* 2008; Stavenuiter *et al.,* 2015).

2. Measuring calcium (Ca) levels and alkaline phosphatase enzyme activity: A calcium colorimetric assay kit (Randox UK) and was used according to the manufacturer's protocol (Stavenuiter *et al.,* 2015).

3. Measurement of phosphate (P) level: Via a colorimetric assay kit (Biovision USA)(Stavenuiter *et al.,* 2015).

4. Alkaline phosphatase enzyme (ALP) activity: An alkaline-phosphatase colorimetric assay kit (Randox UK) was used according to the manufacturer's protocol. The enzyme activity was assessed at 3 time points, and the average was used as the final result (Randox 2015).

Bone specimen and Histology:

After euthanasia of the rats, the right femur bone was used for the studies of bone structure. The bone specimens were fixed for two days in 0.5% cyanuric chloride in methanol containing 1% (0.1 M) N-methylmorpholine (Yoshiki, 1973), decalcified in 10% formic acid formalin and were routinely processed for hematoxylin and eosin (HE) staining. The slides were then examined under a compound light microscope and histopathological changes were assessed. The regions of interest (ROIs) were randomly selected within three sections per limb and viewed under the microscope at 400 × magnification (Iyanda and Iheakanwa, 2014), and digital images of histological sections were taken and analyzed using image J software (Egan *et al.,* 2012) (Image J, National Institutes of Health, USA). Image J tools described by Egan *et al.,* (2) were used to measure osteoid area and areas of bone marrow spaces (trabecular separation) in the selected ROIs. Two ROIs from each sample were selected and analyzed.

Statistical Analysis:

The data were presented as the mean ± SD. Statistical significance before and after was determined by paired Student's "t" test. An independent samples T test was used to compare any groups of interest while an ANOVA with a post hoc test was used to analyze the differences in multiple comparisons. P values < 0.05 were considered to be significant. For the statistical analyses, SPSS version 22 for Windows (SPSS Inc. Chicago, IL, USA) was used.

RESULTS

Bone health in growing rats fed a vitamin D deficient diet:

Fig. 1. shows the histopathological differences between rats fed on a customized vitamin D deficient diet (group II) vs. the rats of the control group (group I). Grosslycut sections revealed no significant histopathological changes. Microscopic examinations revealed moderate osteoporotic changes with reduced

thickening of the bone cortex associated with widely separated bone trabeculae containing bone marrow element in group II rats, while the bone structures in the control group revealed benign bone tissue and trabeculae (figure $1A_{b-c}$). Tools of image J software were used to objectively measure osteoid area and trabecular separation in both groups and revealed a significant reduction of the osteoid area and a significant increase in trabecular separation in ROIs selected from group II *(Group I vs. Group II: 19.82±0.82 vs. 12.48±0.46mm²* and *27.46±0.59 vs. 33.40±0.52 mm²*, respectively, *$P < 0.05$)(fig. 1B).*

Changes in plasma levels of 25 Hydroxyvitamin D3, parathyroid hormone, calcium, phosphate and alkaline phosphatase among all subgroups:

Levels of 25 Hydroxyvitamin D3 in plasma of all subgroups before and after 10 days period of treatment were shown in table 2. Additionally all measured parameters in all subgroups after treatment period were reported in table 3.

Effects of sunlight exposure on vitamin D deficient and vitamin D sufficient rats:

Fig. 2A shows structural changes of the femur of vitamin D deficient rats after 10 days sun exposure. Trabecular thickness and number were improved together with a reduction of bone marrow spaces. Objective measurements of osteoid area and trabecular separation in Sd subgroups before and after sun exposure revealed a significant increase in osteoid area and a significant decrease in trabecular separation (fig. 2B) *(Sd Before vs. Sd After: 13.47±0.35 vs. 20.94±0.17 mm²* and *30.73±0.80 vs. 21.98±0.67 mm²*, respectively, *$P < 0.05$).*

Fig.$1A_{a,b}$ demonstrates an insignificant difference in the histological structure and objective histomorphometric parameters between the vitamin D sufficient subgroup and the vitamin D sufficient-group with sun exposure (Ss subgroup) (figure 1B).

Effects of vitamin D supplementation on vitamin D deficient rats:

In regards to the effect of a given dose of vitamin D on bone structure, microscopic examination revealed some improvement in the moderate osteoporotic picture of before-sections, while the histomorphometric measures of after-ROIs revealed significant increase in osteoid areas *(Tt Before vs. Tt After: 14.75± 0.49 vs. 19.78±0.5, $P < 0.05$)* and an insignificant change in trabecular separation (fig. 3).

Table 2: levels of 25 Hydroxyvitamin D3 in plasma of all subgroups before and after 10 daysperiod of treatment.

Group	Mean ± SD		*P (Paired T test)*
	BEFORE (ng/ml)	AFTER (ug/ml)	
Sd group	14.40±3.19	14.19±2.72	0.680
Tt group	14.33±3.43	14.05±3.08	0.356
Ct group	18.26±6.38	15.59±2.45	0.421
Ss group	35.16±10.54	30.06±13.14	0.300
C group	31.68±10.40	36.84±9.16	0.352

Table 3: Study variables among groups after treatment period presented as means ±SD. One-way ANOVA test with post hoc test was calculated. ** p≤0.05*

Groups		*25 OH Vit D3 (ng/ml)*	*PTH(pg/ml)*	*Calcium(mg/dl)*	*Phosphorus(mg/dl)*	*ALP(U/l)*
Sd group		14.19±2.72	67.69±13.18	6.48±2.12	1.42±0.42	156.14±43.31
Tt group		14.05±3.08	86.05±9.67	5.32±1.28	1.33±0.32	182.62±61.83
Ct group		15.59±2.45	78.93±8.31	6.68±1.92	1.17±0.62	171.00±17.61
Ss group		30.06±13.14	15.56±2.73	9.92±0.48	3.64±0.83	75.96±35.42
C group		36.84±9.16	36.84±9.16	10.24±0.92	3.67±1.13	58.50±11.47
F (between groups)		13.25	59.72	14.05	20.23	12.81
P value		0.000*	0.000*	0.000*	0.000*	0.000*
Tukey HSD	*Sd vs. Ct*	1.000	0.451	1.000	0.996	0.996
	Tt vs. Ct	1.000	0.850	0.532	1.000	0.999
	Sd vs. Tt	1.000	0.029*	0.703	1.000	0.907
	Ss vs. C	0.651	0.011*	0.999	1.000	0.990

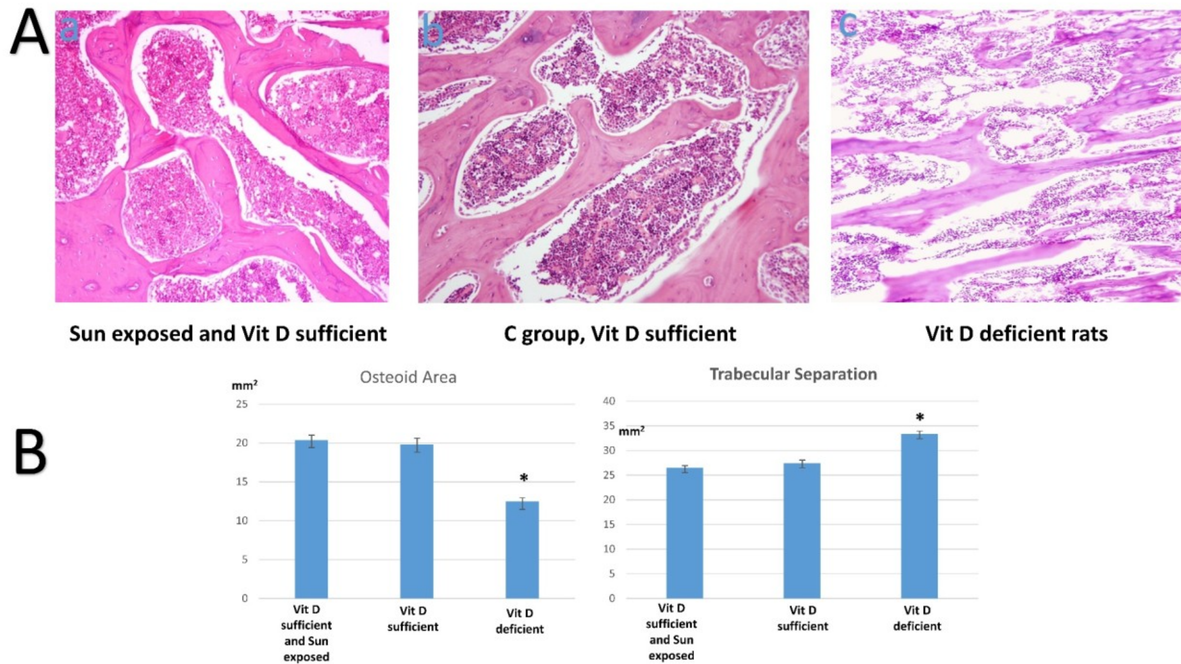


Fig 1: Histopathological bone changes among sun-exposed, vitamin D sufficient subgroup (Aa), vitamin D sufficient control (Ab), and vitamin D deficient subgroup (Ac), in addition tohistomorphometric deference in osteoid area and trabecular separation among them (B). ** p≤0.05.*

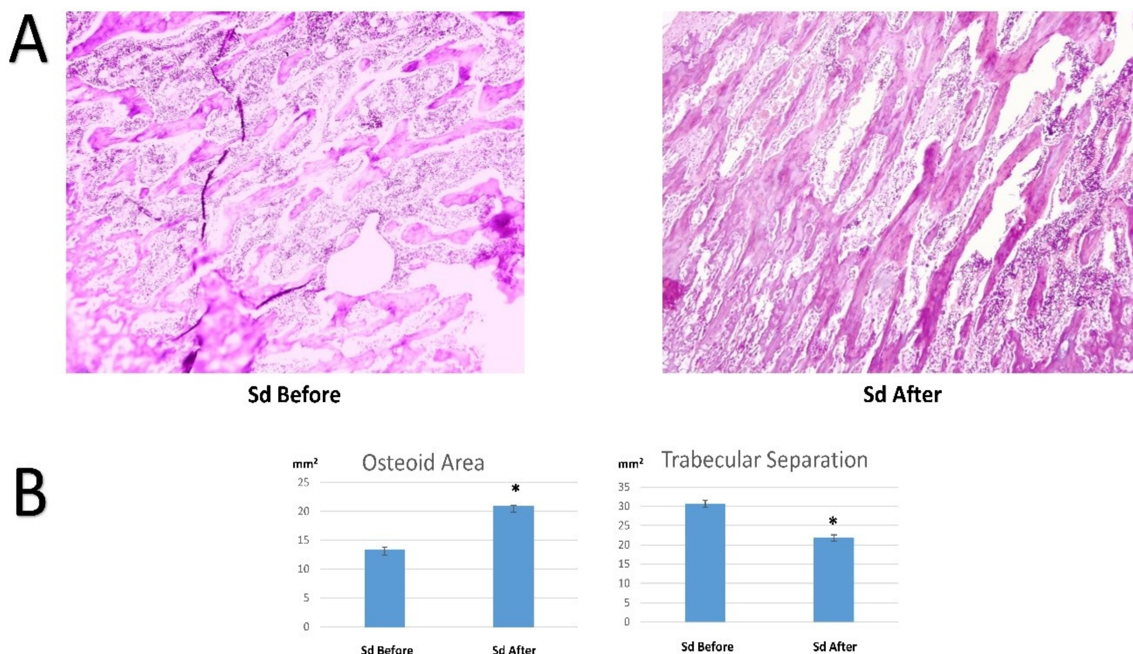


Fig. 2: Histopathological changes in vitamin D deficient rats of Sd subgroup before and after 10 day period of sun exposure (A) and significant improvement in osteoid area and trabecular separation before and after exposure (B). ** p≤0.05*

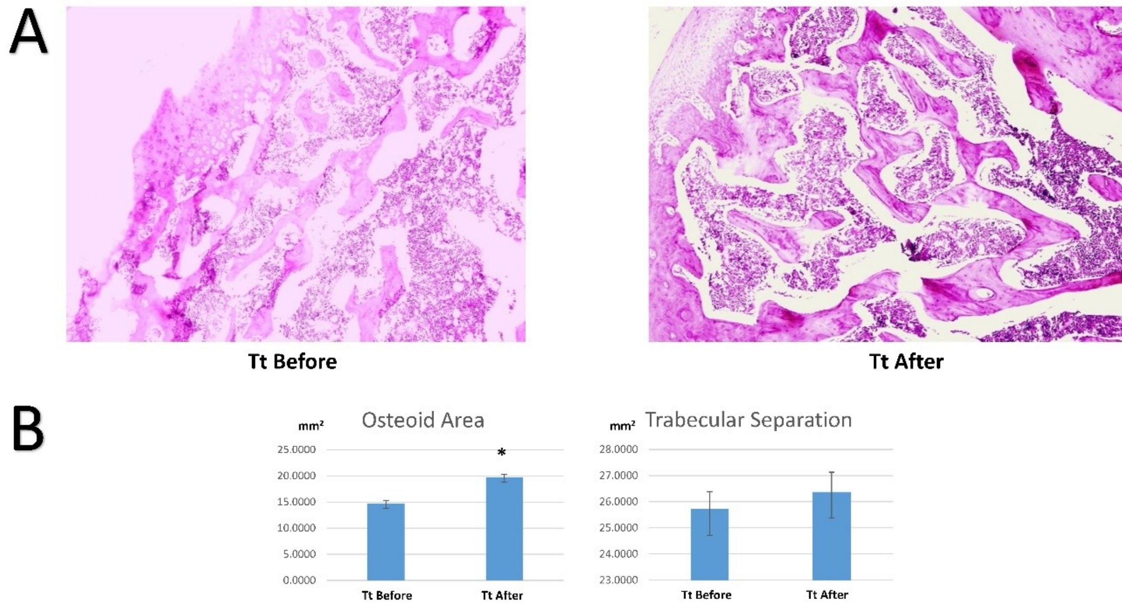


Fig. 3: Histopathological (A) and histomorphometric parameters (B) changes in vitamin D deficient rats of Tt subgroup before and after treatment period. ** p≤0.05*

DISCUSSION

Vitamin D deficiency is a major metabolic syndrome that mainly worsens the bone health in the form of bone growth retardation and the development of classic signs and symptoms of rickets, osteopenia and osteoporosis (Huldschinsky, 1919; Larsen *et al.,* 2004). In this study, a rat model of normocalcemic hypovitaminosis D was utilized by feeding weanling rats on a customized vitamin D deficient diet. All vitamin D deficient subgroups (Sd, Tt & Ct) showed variable degrees of secondary hyperparathyroidism (PTH > 65 pg/ml cutoff value, (Aloia *et al.,* 2006)), normocalccemia (Calcium within the normal range 5.3-13 mg/dl (Johnson-Delaney 1996), hypophosphatemia and increased alkaline phosphatase enzyme activity. In addition, histopathological and histomorphometric measures revealed moderate osteoporotic changes with a significant reduction of bone area and a wide separation of bone trabeculae. These results were partially in line with those of Kollenkirchen *et al.,* (1991), the only difference being normal phosphate and PTH in normocalcemic vitamin D deficient rats in the Kollenkirchen model. The main cause of this difference was dietary lactose, which increases passive intestinalcalcium absorption (Bronner, 1987). Furthermore, these metabolic and histological changes in normocalcemic vitamin D deficient growing rats were similar to those reported by Lester *et al.,* (1982).

The absence of measured of 1, 25 dihydroxyvitamin D levels is a considerable limitation to this study even though current endocrine societies guidelines recommend an assessment of the father metabolite for screening of vitamin D deficiency. Serum levels of 1,25-dihyroxyvitamin D have little or no relation to vitamin D stores but rather are regulated primarily by parathyroid hormone levels, which in turn are regulated by calcium and/or vitamin D (Holick *et al.,*, 2011). The post-interventional insignificant decrease in the level of 25 hydroxyvitamin D in both sun exposed and vitamin D_3 supplemented subgroups may be due to the relatively short treatment period or the consumption of the vitamin D pool in restoring bone structure. The latter explanation is supported by significant positive changes in histological and histomorphometric parameters measured before and after the treatment period. These findings regarding sun exposure were in line with previous studies conducted in Nigeria (Iyanda and Iheakanwa 2014, Iyanda, 2014). These changes were more prominent with the sun-exposed rather than the commercial vitamin D supplemented subgroup.

Hyperparathyroidism was a common finding in all of the subgroups fed a vitamin D deficient diet. Active vitamin D metabolites decrease PTH synthesis in vitro and in vivo (Silver 1985, Silver *et al.,* 1986). It was concluded that 1,25-dihydroxyvitamin D, independent of changes in intestinal calcium absorption and serum calcium, can represses the transcription of PTH by binding to the vitamin D receptor, which heterodimerizes with retinoic acid X receptors to bind vitamin D-response elements within the PTH gene. In addition, 1,25-dihydroxyvitamin D regulates the expression of calcium-sensing receptors to indirectly alter PTH secretion. As a result, reduced concentrations of calcium-sensing and vitamin D receptors and altered mRNA-binding protein activities within parathyroid cells, increase PTH secretion in addition to the more widely recognized changes in serum calcium, phosphorus, and 1α,25-dihydroxy-vitamin D (Kumar and Thompson, 2011). These facts may in part explain of hyperparathyroidism here in this study i.e., a result of low vitamin D metabolites in the plasma of group II rats. Despite normal Ca levels, hypovitaminosis D caused a state of hyperpara-thyroidism, evidencing the relatively narrow range of regulation of PTH secretion by extracellular calcium (Mundy and Guise, 1999). The limited effect of sun light on the level of PTH in the Sd subgroup vs. the Tt subgroup (67.69±13.18 vs. 86.05±9.67, $P < 0.05$) and in Ss subgroup vs. C subgroup (15.56±2.73 vs.36.84±9.16, $P < 0.05$) can be explained in the light of above data by the UVB-induced increase in vitamin D metabolites and calcium sensing. UVB photons with energies of 290-315 nm are absorbed by 7-dehydrocholesterol in the skin and converted to previtamin D_3. Previtamin D_3 undergoes a rapid transformation into vitamin D_3 within the plasma membrane. These changes in vitamin D-molecules could alter membrane permeability and possibly open up a pore to permit the entrance and exit of ions including calcium, ensuring the availability of vitamin D and Ca, leading to a reduction of PTH secretion (Tian *et al.,* 1993; Wacker and Holick, 2013). However, excessive exposure to sunlight does not result in vitamin D intoxication, as both previtamin D_3 and vitamin D_3 are photolyzed to several noncalcemic photoproducts (Holick *et al.,* 2007). This is not the case with pharmacological vitamin D supplementation.

The mean changes in pre- and post-sun exposure 25 hydroxyvitamin D levels were-0.21 ng/mL for the Sd subgroup and -5.10 ng/mL for the Ss subgroup, but they were not statistically significant. These data were consistent with human results reported by Lee *et al.,* (2012), despite the exposure of their study subjects to sunlight for 20 min for four weekends (8 days). This large difference in response of vitamin D deficient rats to sunlight vs. vitamin D sufficient rats is also appear in PTH levels (i.e., insignificant change of PTH between the Sd and the Ct subgroups, while, significant reduction of PTH in the Ss vs. the C subgroups). However, the histological changes were more pronounced in the vitamin D deficient subgroup (figure 2, 3). This demonstrates that vitamin D deficient bones respond in a different manner to sunrays in a manner that may extend beyond vitamin D- and calcium-related actions. In line with this hypothesis, we observed a significant reduction of PTH levels together with an insignificant reduction of alkaline phosphatase activity between the Sd and Tt subgroups.

CONCLUSIONS

In a-conclusion, the effects of sun-exposure on bone health may extend beyond normalization of 25 hydroxy vitamin D plasma levels to a more positive effect on bone structure and the hormones that control bone mass, which may be mediated by other mechanisms.

REFERENCES

Aloia, JF, Feuerman MS and Yeh JK (2006)Reference range for serum parathyroid hormone. *Endocr Pract*, 12(2): 137–144.

Alsuwadia AO, Farag YM, Al Sayyari AA, Mousa DH, Alhejaili FF, Al-Harbi AS, Housawi AA, Mittal BV and Singh AK (2013)Prevalence of vitamin D deficiency in Saudi adults. *Saudi Med J*,34(8):814-8.

Bakhtiyarova S, Lesnyak O, Kyznesova N, Blankenstein MA and Lips P (2006)Vitamin D status among patients with hip fracture and elderly control subjects in Yekaterinburg, Russia. *Osteoporos Int*,17:441– 6.

Bio-Serv, Rodent diet (2015) [Online].USA:Bio-Serv company. Available from: https://www.bio-serv.com/category/Rodent_Diets.html [accessed 20 January 2016]

Bronner F (1987) Calcium absorption. In: Physiology of the GastrointestinaZ Tract (2nd ed.), edited by L. R. Johnson. New York: *Raven,* pp:1419-1435.

Egan KP, Brennan TA and Pignolo RJ (2012) Bone histomorphometry using free and commonly available software. *Histopathology*, 61(6): 1168–1173.

El Rifai NM, Abdel Moety GA, Gaafar HM and Hamed DA (2014) Vitamin D deficiency in Egyptian mothers and their neonates and possible related factors. *J Matern Fetal Neonatal Med*, 27(10):1064-8.

Fleet JC, Gliniak C, Zhang Z, Xue Y, Smith KB, McCreedy R and Adedokun SA (2008) Serum Metabolite Profiles and Target Tissue Gene Expression Define the Effect of Cholecalciferol Intake on Calcium Metabolism in Rats and Mice. *J. Nutr*, 138: 1114–1120.

Fuleihan GE (2009) Vitamin D Deficiency in the Middle East and its Health Consequences for Children and Adults. *Clinic Rev Bone Miner Metab*,7:77–93.

Green RJ, Samy G, Miqdady MS, El-Hodhod M, Akinyinka OO, Saleh G, Haddad J, Alsaedi SA, Mersal AY, Edris A and Salah M (2015) Vitamin D deficiency and insufficiency in Africa and the Middle East, despite year-round sunny days. *S Afr Med J*, 105(7):603-5.

Holick MF (2002) Vitamin D: the underappreciated D-lightful hormone that is important for skeletal and cellular health*. Curr.Opin.Endocrinol Diabetes*, 9:87-98.

Holick MF (2004) Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr*, 80 (suppl) :1678S– 88S.

Holick MF and Chen TC (2008) Vitamin D deficiency: a worldwide problem with health consequences. *Am J ClinNutr*. 87(suppl):1080S– 6S.

Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH and Weaver CM (2011) Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*, 96(7):1911-30.

Holick MF, Chen TC, Lu Z and Sauter E (2007) Vitamin D and skin physiology: a D-lightful story. *J Bone Miner Res*, 22(Suppl 2):V28–33.

Holick MF, Shao Q, Liu WW and Chen TC (1992) The vitamin D content of fortified milk and infant formula. *N Engl J Med*, 326:1178-1181.

Huldschinsky K (1919) Ultraviolet irradiation of rachitic children. *Deutsche Med Wochenschr*, 45:712–3.

Iyanda AA and Iheakanwa CI (2014) Tissue histology and antioxidant defense system in female rats exposed to sunrays. *IJBMSP*, 4:5-8. ISSN: 2049-4963.

Iyanda AA (2014) Exposure to Sun Rays: An Investigation of Serum Micronutrient Status in Wistar Rats. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 04 (30); 1-5.

Johnson-Delaney C (1996) Exotic Animal Companion Medicine Handbook for Veterinarians. USA: Zoological Education Network, pp:356.

Kollenkirchen U, Walters MR and Fox J (1991) Plasma Ca influences vitamin D metabolite levels as rats develop vitamin D deficiency. *Am J Physiol*, 260(3 Pt 1):E447-52.

Kumar R and Thompson JR (2011) The regulation of parathyroid hormone secretion and synthesis. *J Am SocNephrol*, 22(2):216-24

Larsen ER, Mosekilde L and Foldspang A (2004) Vitamin D and calcium supplementation prevents osteoporotic fractures in elderly community dwelling residents: a pragmatic population-based 3-year intervention study. *J Bone Miner Res,*19:370–8.

Lee S, Park S, Kim K, Lee D, Kim W, Park R and Joo N (2012) Effect of Sunlight Exposure on Serum 25-Hydroxyvitamin D Concentration in Women with Vitamin D Deficiency: Using Ambulatory Lux Meter and Sunlight Exposure Questionnaire. *Korean J Fam Med*, 33:381-389.

Lester GE, Vanderwiel CJ, Gray K and Talmage RV (1982) Vitamin D deficiency in rats with normal serum calcium Concentration. *Proc. Natl. Acad. Sci*. 79:4791-4794.

Mundy GR and Guise TA (1999) Hormonal Control of Calcium Homeostasis. *Clinical Chemistry,* 45:8(B)1347–1352.

Randox, alkaline phosphatase enzyme(2015) [Online].UK: Randox. Available from:http://www.randox.com/ alkaline-phosphatase/[accessed 1 January 2016]

Reeves PG, Nielsen FH and Fahey GC (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr*, 123:1939–51.

Shuai B, Shen L, Yang YP, Xie J, Zhou PQ, Li H, Guo XF, Zhao J and Wu JL (2008)Effects of Chinese kidney-tonifying drugs on bone mineral density (BMD), biomechanics, 25-hydroxy vitamin D3 and 1,25-dihydroxy vitamin D3 of ovariectomized osteoporosis rats. *Zhongguo Gu Shang*, 21(11):850-3.

Silver J, Naveh-Many T, Mayer H, Schmelzer HJ and Popovtzer MM (1986) Regulation by vitamin D metabolites of parathyroid hormone gene transcription in vivo in the rat. *J Clin Investig*,78:1296-1301.

Silver J, Russell J and Sherwood LM (1985) Regulation by vitamin D metabolites of messenger RNA for preproparathyroid hormone in isolated bovine parathyroid cells. *Proc Natl AcadSci USA*, 82:4270-4273.

Stavenuiter AW, Arcidiacono MV, Ferrantelli E, Keuning ED, Cuenca MV, Wee PM, Beelen RH, Vervloet MG and Dusso AS (2015) A Novel Rat Model of Vitamin D Deficiency: Safe and Rapid Induction of Vitamin D and Calcitriol Deficiency without Hyperparathyroidism. *BioMed Research International*, Article ID 604275, 5 pages http://dx.doi.org/10.1155/2015/604275.

Tangpricha V, Koutkia P, Rieke SM, Chen TC, Perez AA and Holick MF (2003) Fortification of orange juice with vitamin D: a novel approach to enhance vitamin D nutritional health. *Am J ClinNutr*, 77:1478-1483.

Tian XQ, Chen TC, Matsuoka LY, Wortsman J and Holick MF (1993) Kinetic and thermodynamic studies of the conversion of previtamin D_3 to vitamin D_3 in human skin. *J BiolChem*.,268:14888–92.

Wacker M and Holick MF (2013)Sunlight and Vitamin D A global perspective for health. *Dermatoendocrinol*. 5(1): 51–108.

Yoshiki S (1973) A simple histological method for identification of osteoid matrix in decalcified bone. *Stain Technol*., 48: 223–38.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Observation on the histochemistry of the developing ova in *Haemonchus contortus* (Nematoda)

Jatinderpal Singh

Department of Zoology, Baring Union Christian College, Batala-143505 (India)
Email: doctorjatinder@yahoo.com

Manuscript details:

Received: 08.02.2016
Accepted: 08.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Singh Jatinderpal (2016) Observation on the histochemistry of the developing ova in *Haemonchus contortus* (Nematoda). *International J. of Life Sciences*, 4(1): XX-XX.

Acknowledgement
The author extends sincere gratitude to Prof. (Dr.) Manjit Johal for her guidance, encouragement and constant support for this research work. The author is also indebted to the Professor and Head, Department of Zoology, Punjabi University, Patiala and Principal, Baring Union Christian College, Batala for providing necessary facilities to carry out the research.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

In *Haemonchus contortus*, the concentration of various metabolites differ in various stages of oogenesis. Though an adequate quantity of carbohydrates is evidenced in ovarian epithelium, perinuclear spaces of oogonia, oocytes and rachis but the protoplasmic processes connecting the oogonia to the rachis are completely devoid of the same. Developing oocytes imbibe large concentrations of glycogen from the ovarian epithelium and subsequently use it for the formation of chitinous layer of the egg shell. The mature ova gets surrounded by an additional resistant layer of acid mucopolysaccarides. High nucleic acid activity has been detected in both oogonia as well as oocytes and fertilized ova show a spurt of ribosomes in them. The secondary oocytes are full of proteinaceous granules and show an intense activity of RNA indicating the occurrence of rapid protein synthesis at this stage. The lipids seem to be a major constituent of the egg shell envelope of the fertilized ova.

Keywords: Histochemistry, oogenesis, egg shell, Nematoda, *Haemonchus contortus*.

INTRODUCTION

Haemonchus contortus is a serious nematode parasite of sheep (*Ovis aries*) and goat (*Capra hircus*) of cosmopolitan distribution. It causes severe anaemia resulting in weight loss, poor milk yield and wool production. Medium infection causes sheep to lose condition and heavy infection may result into death. Thousand of worms may occur in a single ruminant stomach and it has been estimated that 4000 worms suck about 63 cm^3 of blood per day (Smyth, 1996). Baker *et al*. (1956) have estimated that a single worm causes an average daily loss of 0.08 ml. of blood.

Previously, the histomorphology and histochemistry of various organ systems of *Haemonchus contortus* was studied by Singh and Johal (1997), Singh (2000), Singh and Johal (2001a; 2001b; 2001c), Singh and Johal (2004) and Singh (2015a; 2015b; 2015c, 2015d; 2015e). The present research paper describes the histochemical aspect of developing ova in female *Haemonchus contortus*, which can fill the hitherto existing gaps in

information regarding this aspect. The earlier literature on histochemistry of nematode parasites reveals that both the reproductive tract as well as the developing gametocytes shows a variable spectrum of distribution of metabolites viz. glycogen, proteins and lipids. The present histochemical localization of macromolecules will be of significance in understanding the metabolic activities and fundamental functional aspects of the organs of this nematode. This study will fill the hitherto existing gaps in information regarding this aspect in nematodes and also form a basis for the development of effective chemotherapeutic measures against this serious pathogenic parasite of domestic ruminants.

MATERIALS AND METHODS

The adult female worms were extracted from the abomasum portion of stomach of sheep (*Ovis aries*). In order to remove debris, the nematode worms were washed in 0.85% NaCl solution. For histochemical studies, the worms were fixed in alcoholic Bouin's fixative and Carnoy's fixative, dehydrated in a graded series of alcohol, cleared in methyl benzoate and embedded in paraffin wax. The sections were cut at 7µm in transverse and longitudinal planes by using rotary microtome. The serial sections arranged on albuminised slides were stained.

General carbohydrates were studied by Periodic acid Schiff's staining technique (McManus, 1948). Glycogen was detected histochemically by Best's carmine staining (Best, 1906) and acid mucopolysaccharides by Alcian blue (Steedman, 1950). Nucleic acids were detected by Gallocyanin chromalum (Einarson, 1951) and Methyl green pyronin Y (Kurnick, 1955) techniques. For the localization of proteins, Mercuric bromophenol blue staining (Bonhag, 1955) and Ninhydrin Schiff's staining (Yasuma and Ichikawa, 1953) were used. The histochemical presence of lipids was detected by Sudan black B staining (McManus, 1946) and Oil red O in isopropanol (Lillie and Ashburn, 1943). The slides were examined under the microscope and photo micrographed.

RESULTS AND DISCUSSION

In female *Haemonchus contortus*, carbohydrates are localized in all the developing stages of germ cells. An adequate quantity of carbohydrates is found in the oogonia and oocytes present in the ovary as evidenced by Periodic acid Schiff's staining (Figs. 1 and 2). Glycogen is seen in the perinuclear spaces of oogonia, oocytes and the wall of rachis, whereas the protoplasmic processes connecting the two are devoid of it (Fig. 4). In the secondary oocytes, the carbohydrates get more concentrated in the cytoplasmic portion (Figs. 2 and 3). The spermatozoa lying in the seminal receptacle show a substantial amount of glycogen, whereas in the fertilized ova glycogen is concentrated in the chitinous layer and the egg yolk has only a lesser amount of it (Fig. 5 and 6).

Both structural as well as cytoplasmic proteins are found in abundance in the developing stages of ova of *Haemonchus contortus*. The proliferating oogonia in germinal zone of ovaries show a profuse and rachis a moderate concentration of proteins (Figs. 7 and 8). In the developing oocytes, the protein granules are spread over in the cytoplasmic region, whereas the nuclear spaces are clear of it except the presence of some condensed chromatin granules (Fig. 9). The mature ova lying in the uterus are full of proteinaceous ribosomes and the outer uterine layer of egg shell has protein as its main constituent (Figs. 10, 11 and 12). Near the vaginal region the two-celled stage of fertilized ova have an intense concentration of protein granules in the egg yolk. The chromosomes of dividing nuclei and outer shell wall are also positive for proteins (Fig. 13).

A substantial amount of nucleic acids is observed in the developing stages of the ova. The nucleic acids are seen restricted to only the nuclear region of the oogonia indicating DNA activity at this stage, as evidenced by Gallocyanin chromalum staining (Fig. 14). In the secondary oocytes, the nucleus as well as cytoplasm stains intensely with Gallocyanin chromalum revealing the presence of both DNA and RNA activity in these stages of ova (Figs. 15, 16 and 17). The absence of cytoplasmic RNA in rapidly dividing oogonia indicate the absence of protein synthesis at this site and on the contrary the high amount of protein in the cytoplasmic area along with the presence of cytoplasmic RNA in the growing oocytes suggests that intense protein synthesis is taking place in this area of the developing ova (Figs 9, 14 and 16).

The oolemma surrounding the secondary oocytes is lipid positive and the lipid granules are seen distributed throughout the cytoplasmic region in high

concentration (Fig. 18). Furthermore, the lipid seems to be the major constituent of the egg shell envelope of fertilized ova, whereas its content is comparatively less in the cytoplasm (Figs. 19 and 20). The presence of acid mucopolysaccharides is only seen the egg envelope of the mature ova (Fig. 21). No evidence of this constituent is seen in earlier developing stage of oogonia and oocytes.

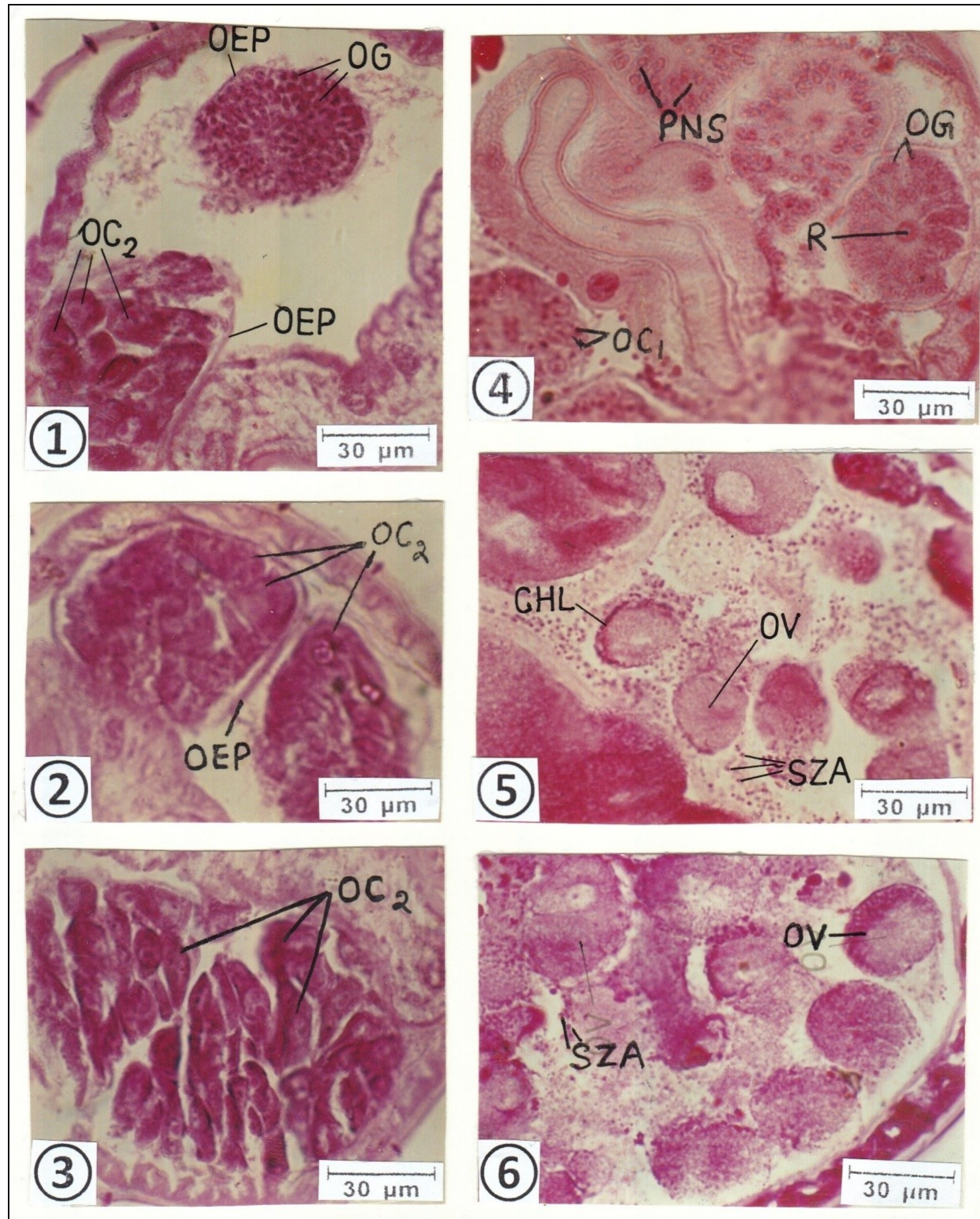


Fig. 1 - 6: *Haemonchus contortus.*

Fig. 1, 2 and 3: A Portion of Transverse Sections (T.S.) of female through ovary showing concentration of carbohydrates in the various stages of oogonia and oocytes (Periodic acid Schiff's staining);
Fig. 4, 5 and 6: A Portion of Transverse Sections (T.S.) of female showing concentration of glycogen in the developing stages of ova (Best's carmine staining).

Abbreviations used: PNS: Perinuclear Spaces of Oogonia; OEP: Ovarian Epithelium; OC_1: Primary Oocytes; OC_2: Secondary Oocytes; OG: Oogonia; OV: Fertilized Ova; R: Rachis; SZA: Spermatozoa fertilizing ova; CHL: Chitinous layer of the egg shell.

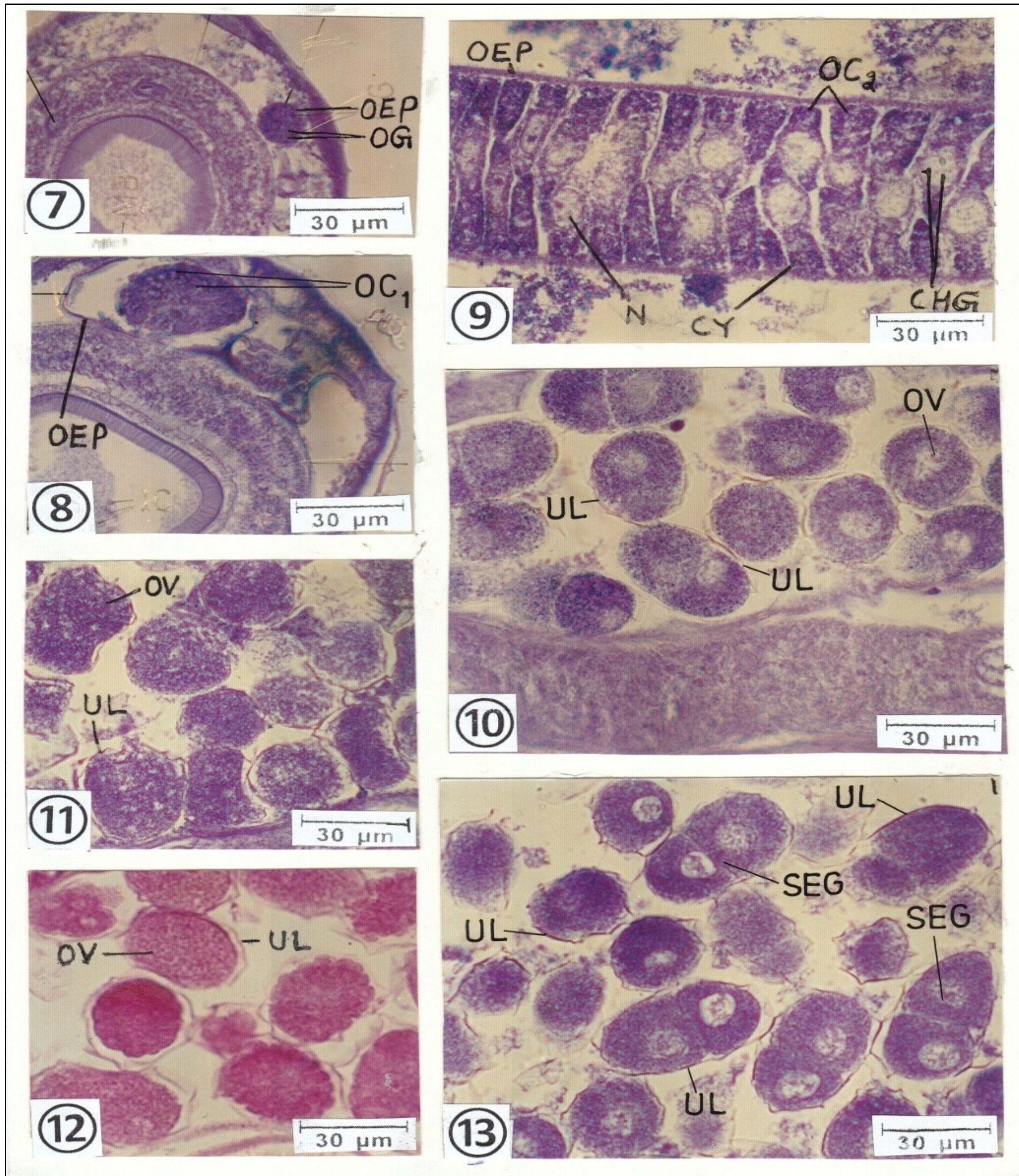


Fig. 7 - 13: ***Haemonchus contortus.***

Fig. 7 and 8: A Portion of T.S. of female through germinal zone of ovary showing concentration of proteins in oogonia and oocytes (Mercuric bromophenol blue staining);

Fig. 9: A Portion of L.S. of female through growth zone of the ovary showing distribution of proteins in secondary oocytes (Mercuric bromophenol blue staining);

Fig. 10 and 11: A Portion of L.S. through the uterus of the female showing concentration of proteins in the uterine layer of the ovum (Mercuric bromophenol blue staining);

Fig. 12: A Portion of L. S. of female showing concentration of $–NH_2$ proteins in the uterine layer of the ovum (Ninhydrin Schiff's staining);

Fig. 13: A Portion of L. S. of female showing distribution of proteins in the segmented stage of the fertilized ova (Mercuric bromophenol blue staining).

Abbreviations used: CHG: Chromatin Granules; CY: Cytoplasm; OEP: Ovarian Epithelium; OC_1: Primary Oocytes; OC_2: Secondary Oocytes; OG: Oogonia; OV: Fertilized Ova; SEG: Segmented Stage of fertilized ova; UL: Uterine Layer of the egg shell.

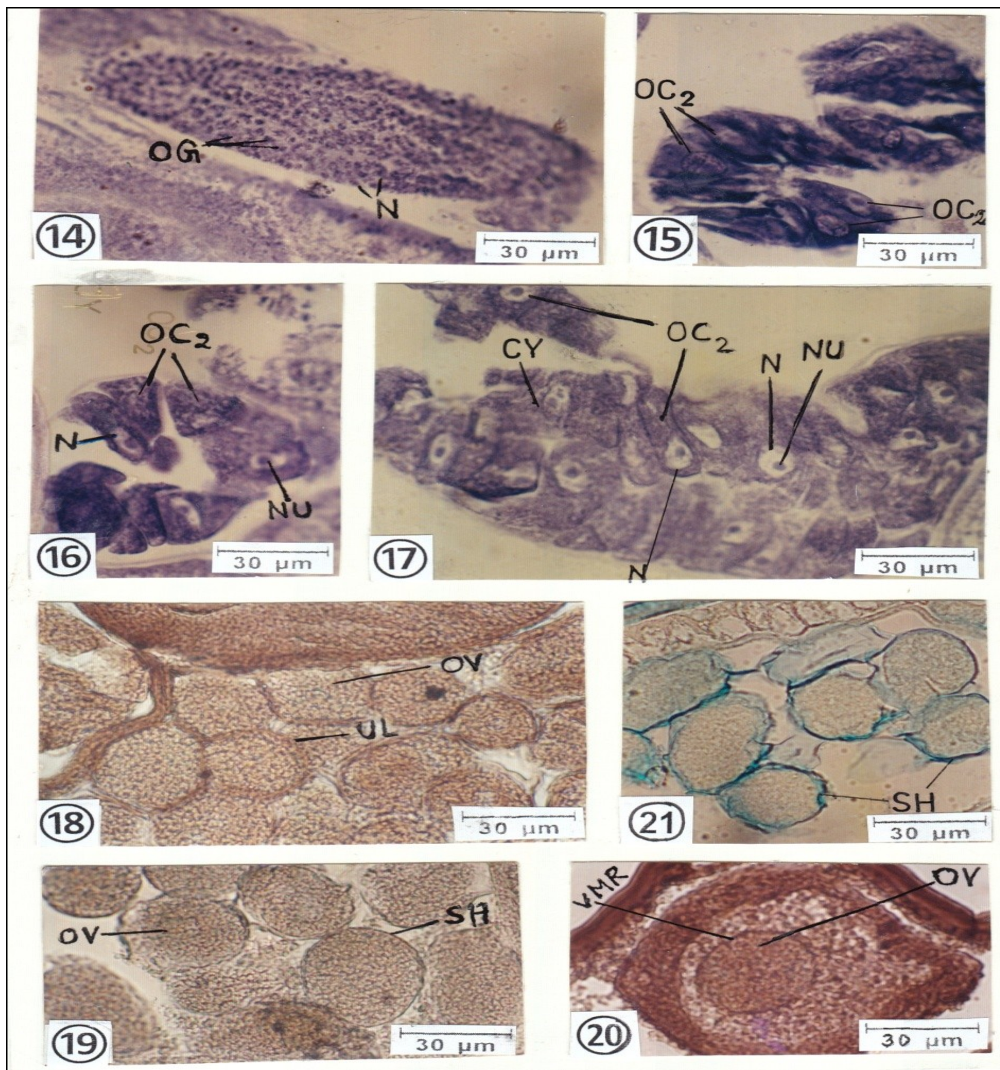


Fig. 14- 21: *Haemonchus contortus.*

Fig. 14: A Portion of L.S. of female through germinal zone of ovary showing concentration of nucleic acids in oogonia (Gallocyanin chromalum staining);
Fig. 15, 16 and 17: A Portion of L.S. of the female through the growth zone of ovaries showing concentration of nucleic acids in the oocytes (Gallocyanin chromalum staining);
Fig. 18, 19 and 20: A Portion of T. S. of female showing concentration of lipids in the fertilized ova (Oil red O in isopropanol staining);
Fig. 21: A Portion of L. S. of female showing concentration of acid mucopolysaccharides in the mature and fertilized ova (Alcian blue staining).
Abbreviations used: CY: Cytoplasm; N: Nucleus; NU: Nucleolus; OC_2: Secondary Oocytes; OG: Oogonia; OV: Fertilized Ova; SEG: Segmented Stage of fertilized ova; SH: Egg Shell of ovum; UL: Outer Uterine Layer; VMR: Vitelline Membrane of the fertilized ovum.

Histochemical studies reveal that the concentration of various metabolites varies during different stages of oogenesis. Faure-Fremiet (1913) has negated the presence of glycogen in the dividing oogonia of *Parascaris equorum*. Lee (1960) has described that in *Thelastoma bulhoesi,* the amount of glycogen in oogonia increases along the length of the ovary. Anya (1964a), Weber (1987) and Johal and Joshi (1993) confirm the findings of the first author and have noted a complete absence of glycogen in the oogonia of *Aspiculuris tetraptera, Loa loa and Trichuris ovis* respectively. Fairbairn (1957), Anya (1964b), Adamson (1983) and Mackinnon (1987) have accounted for a progressive increase in carbohydrate contents in the developing oocytes with a simultaneous depletion of glycogen from the ovary. In *Oesophagostomum columbianum* too, Johal (1995) has reported an increase in the amount of carbohydrates

in the developing oocytes lying in the ovary. In present study on *Haemonchus contortus,* the oogonia possess an adequate quantity of carbohydrate especially in their perinuclear spaces. In the secondary oocytes, a rich concentration of carbohydrate is seen in the cytoplasmic portion. The glycogen aggregated in the oocytes is later used in the endogenous formation of the chitinous layer of the fertilized ova. Thereafter, the ova reveal a little amount of glycogen in the egg yolk.

Fairbairn (1957), Anya (1964a), Wharton (1979) and Adamson (1983) have recorded the complete absence of proteins in the proliferating nematode oogonia. They also reported that the occytes accumulate large quantities of protein during their migration down the ovary. In the oocytes the protein is present in the form of hyaline granules. In the present study on *Haemonchus contortus*, the proliferating oogonia show a profuse quantity of protein and even the cytoplasmic strands connecting them are moderately proteinaceous and the protein is seen spread over whole of the cytoplasmic region of the developing oocytes in the form of granules.

In *Trichuris ovis* (Johal and Joshi, 1993) and *Oesophagostomum columbianum* (Johal, 1995), the proliferating oogonia show a significant concentration of DNA as well as RNA in the nucleus. In present study on *H. contortus*, the nucleic acids are restricted to the nuclear region of the oogonia indicating an intense DNA activity in their rapidly dividing stages. Both the nuclei as well as cytoplasm reveal intense nucleic acid activity in the developing oocytes. Their cytoplasm also reveal a rich amount of protein along with RNA indicating that this area is metabolically active.

Adamson (1983) has reported a large amount of lipid droplets in the developing oocytes of *Ascaris lumbricoides*, Mackinnon (1987) too, demonstrated that the large granules located in the cytoplasm of developing oocytes are lipoidal in nature. In the oocytes of *Toxocara canis*, lipid in the form of drops is described by Brunanska (1997). In *H. contortus*, a high concentration of the lipid granules is found in the cytoplasmic region of the secondary oocytes and the oolemma surrounding them is also lipoidal in nature

In egg shell formation in *Haemonchus contortus* the first vitelline layer gets demarcated in the fertilized ova, this is accompanied by a simultaneous shift of glycogen granules towards the periphery which get concentrated to form the second or the chitinous layer of the eggshell, endogenously. All the previous authors working on oogenesis are in consonance about the endogenous formation of the chitinous layer.

About the outer coat of the egg shell it was earlier established by Faure-Fremiet (1913), Wharton (1915), Chitwood (1931), Jacobs (1950) and Anya (1964a; 1964b) that it is proteinaceous in nature and is formed from the secretions of the uterine cells. The research work of Johal (1995) and Johal and Joshi (1993) reveals that the deposition and composition of the outer uterine layer differs in different species. In *Oesophagostomum columbianum*, thick jelly like lipoproteinaceous stands emerge out from the uterine wall and form a loose network around the fertilized ova. Later their interconnections are broken down, resulting in the formation of loose envelops around the ova which become compact as the ova roll down the uterus (Johal, 1995). In *Trichuris ovis* (Johal and Joshi, 1993), the uterine wall secretes a granular secretion and the fertilized ova press to the uterine epithelium to get coated by the secretion which condenses to form a thick layer. The uterine layer is present only on the sides of the ova leaving the polar plugs uncoated. In the present study on *Haemonchus contortus*, an enormous quantity of secretory granules are shed into the lumen of the uterus which align around the fertilized ova in loose granular envelops. The granules subsequently condense to form regular outer wall of the egg shell which is lipoproteinaceous in nature.

The above facts indicate that the oogonia possess protein and lipid in their active phase of division. The secondary oocytes accumulate large quantities of carbohydrates which are later used up in the formation of chitinous layer, whereas the protein and lipid imbibed, mainly from the yolk granules. The uterine lipid and proteins contribute to the formation of outer layer of egg shell which gets coated by acid mucopolysaccharides in the last portion of the uterus.

REFERENCES

Adamson ML (1983) Ultrastructural observations on the oogenesis and shell formation, *Gyrinicola batrachiensis* (Walton, 1929) (Nematoda: Oxyurida). *Parasitology*, 86 (3) : 489-491.

Anya AO (1964a) Studies on the structure of female reproductive system and egg shell formation in *Aspiculuris tetraptera* Schulz (Nematoda: Oxyuroidea) *Parasitology*, 54 : 699-719.

Anya AO (1964b) The distribution of lipids and glycogen in some female oxyuroids.*Parasitology*, 54 : 555-566.

Baker NF, Allen PH, Longhurst WM and Douglas JR (1956) *American Journal of Veterinary Research*, 20:409-413. Cited from: *Nematode Parasites of domestic animals of man*, N.D. Levine (1968), Burgess Publishing company, Minneapolis, pp 224.

Best F (1906) *Z. Wiss. Mirk.* 23 : 319. Cited from : *Histochemistry, Theoretical and Applied*. A.G. E. Pearse (ed.), J and Churchill, London (1968).

Bonhag PF (1955) *J. Morphol.* 96 : 381. Cited from : *The Structure of Nematodes*, A. F. Bird (1971), Academic Press, New York and London.

Brunanska M (1997) *Toxocara canis* (Nematoda: Ascarididae): The fine structure of the oviduct, oviduct-uterine junction and uterus. *Folia Parasitologica*, 44: 55-61.

Chitwood BG (1931) A comparative histological study of certain nematodes. *Ztoshr. Morphol. & Oekol. Tiere.* 23(1, 2) : 234-284.

Einarson L (1951) *Acta Pathol. Microbiol. Scand*., 28: 82. Cited from: *The Structure of Nematodes,* A. F. Bird (1971), Academic Press, New York and London.

Fairbairn D (1957) The biochemistry of *Ascaris*. *Experimental Parasitology,* 6 : 491-554.

Faure-Fremiet E (1913) *La formation de la membrance de 1' Oeufd' Ascaris megalocephala. Compt. Rend.Soc. Biol., Paris,* 74: 567-569.

Jacobs L (1950) Nemic ova: the chemistry of the egg membrane. In: *An Introduction to Nematology*, B.G. Chitwood (ed.) Monumental Printing Co., Baltimore, Maryland, pp 186-187.

Johal M (1995) Histochemical aspect of developing ova in *Oesophagostomum columbianum* (Nematoda). *Journal of Parasitology and Applied Animal Biology*. 4(1) : 37-41.

Johal M and Joshi A (1993) Histochemical studies on the female reproductive organs of *Trichuris ovis* (Nematoda). *Current Nematology*, 4(2) : 219-224.

Kurnick NB (1955) Stain technology, 30: 213-230. Cited from: *Histological and Histochemical methods,* J. A. Kiernan (ed.), Pergamon Press (1981), Oxford.

Lee DL (1960) The distribution of glycogen and fat in *Thelastoma bulhoesi*, a nematode parasitic in cockroaches. *Parasitology*, 50 : 247-259.

Lillie RD and Ashburn IL (1943) Supersaturated solution of fat stains in dilute isopropanol for demonstration of acute fatty degeneration not shown by Herztieimer technique. *Arch. Pathol*., 36 : 432.

Mackinnon BM (1987) An ultrastructural and histochemical study of oogenesis in the trichostrongylid nematode *Heligmosomoides polygyrus. Journal of Parasitology*, 73(2): 390-399.

McManus JFA (1948) Cited from: *The Structure of Nematodes*, A. F. Bird, Academic Press, New York and London.

McManus JPA (1946) In: *Histochemistry: Theoretical and Applied.* A.G. Pearse (ed.), J.A. Churchill Ltd., London.

Singh J (2000) Histomorphological and histochemical studies of some organ-systems and *in vitro* effect of neem leaf extract on *Haemonchus contortus* (Rudolphi,1803). Ph.D. Thesis, Punjabi University, Patiala.

Singh J (2015a) Histochemical and Histoenzymatic studies on the intestinal epithelium of *Haemonchus contortus* (Nematoda). *International Journal of Life Sciences*, 3(4):325-332.

Singh J (2015b) Histochemical observations on the oesophagus of *Haemonchus contortus* (Nematoda). *Current Nematology*, 26(1, 2):13-16.

Singh J (2015c) *In vitro* effect of neem leaf extract on various organ-systems of *Haemonchus contortus* (Nematoda). *Uttar Pradesh Journal of Zoology,* 35 (2):161-168.

Singh J (2015d) Histomorphology of the copulatory apparatus of male *Haemonchus contortus* (Nematoda). *Journal Punjab Academy of Sciences*, 13-14: 69-71.

Singh J (2015e) Histochemical observations on the genital epithelium and developing gametes in *Haemonchus contortus* (Nematoda). *Journal Punjab Academy of Sciences*, 13-14: 32-35.

Singh J and Johal M (1997) A study on spermatogenesis in a nematode, *Haemonchus contortus. Trends in Life Sciences.* 12 (2):81-86.

Singh J and Johal M (2001a) Structure of the excretory system of adult *Haemonchus contortus* (Nematoda). *Current Nematology*, 12(1, 2):69-72.

Singh J and Johal M (2001b) Structural variations in the genital epithelium of male *Haemonchus contortus* (Nematoda). *Bionature,* 21 (2):77-83.

Singh J and Johal M (2001c) Observations on the foregut (stomodaeum) of *Haemonchus contortus* (Rud., 1803). *Uttar Pradesh Journal of Zoology,* 21 (2):139-145.

Singh J and Johal M (2004) Histological study on the intestine of *Haemonchus contortus* (Rud., 1803). *Journal of Parasitology and Applied Animal Biology*, 13 (1, 2):13-24.

Smyth JD (1996) *Animal Parasitology*, Cambridge University Press, New York and Melbourne, pp 549.

Steedman HF (1950) Alcian blue 8 G.S.: a new stain for mucin. *Quart. J. Micro.Sci.,* 91: 477.

von Kemnitz G (1912) Die Morphologie des Stoffwechsels bei *Ascaris lumbricoides*. *Arch. Zell. Forsch*., 7 : 463-603.

Weber P (1987) The fine structure of the female reproductive tract of adult of *Loa loa. International Journal for Parasitology,* 17 (4) : 927-934.

Wharton LD (1915) The development of eggs of *Ascaris lumbricoides. Philipp. J. Sci. B*., 10 : 19-23.

Wharton DA (1979) Oogenesis and egg shell formation in *Aspiculuris tetraptera* Schulz (Nematoda : Oxyuroidea). *Parasitology*, 78 : 131-143.

Yasuma A and Itchikawa T (1953) *J. Lab. Clin. Med*., 41: 296, Histochemical Techniques, Bancroft, J.D. (1975).

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Histological structure of pancreas in normal control, diabetic control and extract treated *Albino* rats

Kinkar Shobha Bhanudas[1] and Patil Kishor Gopal[2]*

[1]PGT Department of Zoology, RTM Nagpur University, Nagpur (M.S.) India PIN-4400010.
[2]Department of Zoology, Government Institute of Science, R. T. Road Civil Lines, Nagpur (M.S.) India PIN-440 001.
*Corresponding author E-mail- drkgpatil@gmail.com

Manuscript details:

Received: 02.02.2016
Accepted: 08.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Kinkar Shobha Bhanudas and Patil Kishor Gopal (2016) Histological structure of pancreas in normal control, diabetic control and extract treated *Albino* rats, *International J. of Life Sciences*, 4(1): 78-82.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

The present study is carried out to investigate the structure of pancreas in normal control, diabetic control and *Tinospora cordifolia* extract treated albino rats. Healthy albino rats (9 months old) of both the sexes, weighing 150-190gm were used for the experiment. Rats were treated with *Tinospora cordifolia* whole plant extract 20ml/kg body weight, twice a day from day 2 to 30. It was observed that the cells in the pancreas in normal control animals are densely packed. In case of alloxan treated group the cell density significantly diminish. Where as in case of *Tinospora cordifolia* extract treated group it was observed that the empty spaces in islets created by the alloxan induced necrosis is significantly decreased.

Keywords: Insulin, diabetes, *Tinospora cordifolia*, albino rats.

INTRODUCTION

Present study is carried out on albino rat *Rattus norvegicus* due to its metabolic relatedness with human. von Mering and Minkowski found that removing the pancreas from dogs resulted in fatal diabetes, providing the first clue that the pancreas plays a key role in regulating glucose concentrations (von Mering and Minkowski,1890; Brogard *et al*., 1992). In 1910, Edward Albert Sharpey-Schafer hypothesized that diabetes was due to the deficiency of a single chemical produced by the pancreas; he called this chemical insulin, from the Latin word *insula*, meaning island and referring to the pancreatic islet cells of Langerhans. In 1921, Banting and Best actually discovered insulin when they reversed diabetes that had been induced in dogs with an extract from the pancreatic islet cells of healthy dogs (Banting *et al*., 1991 and Bliss 2007). Together with Collip and Macleod, they purified the hormone insulin from bovine pancreases and were the first to use it to treat a patient with diabetes.

Management of diabetes without any side effects is still a challenge in the medical field, as presently available drugs for diabetes have one or more

adverse effects. Since the existing drugs for the treatment of diabetes mellitus do not satisfy our need completely, the search for new drugs continues. In recent years, herbal remedies for the unsolved medical problems have been gaining importance in the research field. Thus, this study was undertaken to explore the efficacy of anti-diabetic activity of *Tinospora cordifolia* in diabetic rats.

It is estimated that 366 million people had diabetes mellitus in 2011; by 2030 this would have risen to 552 million (Brussels, 2011). The number of people with type 2 diabetes mellitus is increasing in every country with 80% of people having diabetes mellitus living in low and middle income countries. Diabetes mellitus caused million deaths in 2011. It is estimated that 439 million people would have type 2 diabetes mellitus by the year 2030. The incidence of type 2 diabetes mellitus varies substantially from one geographical region to the other as a result of environmental and lifestyle risk factors (Zimmet *et al*., 2001). The International Diabetes Federation estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025 (Sicree *et al.,*2006). A study done in Small Township in south India reported a prevalence of 5% (Ramachandran *et al.,* 1988).

The serum insulin level decreased significantly in the alloxan treated group. In the *Tinospora cordifolia* whole plant extract treated group the insulin level reverse to normal. The hike in insulin secretion observed in this study from diabetic control to the normal as observed in *Tinospora cordifolia* treated group indicates that the whole plant extract may exerts its healing effect on the islets of Langerhans (Kinkar and Patil, 2015; 2016).

MATERIALS AND METHODS

Tinospora cordifolia is a large, glabrous, deciduous climbing shrub native to India. The stems are rather succulent with long filiform fleshy aerial roots from the branches. The plant material was collected from hygienic places from in and around the Nagpur city, Soxhlet apparatus was used for the preparation of concentrated extract of *Tinospora cordifolia*. The plant material *Tinospora cordifolia* was collected from hygienic places from in and around the Nagpur city and identified. The collected plant material was washed with water in order to make it free of dirt and other impurities and was shade dried. Shade dried whole Plant material was grind with mortar and pastle into the fine powder and alcoholic and aqueous extract of *Tinospora cordifolia* was prepared according to the standard procedure.

Healthy albino rats (9 months old) of both the sexes , weighing 150-190gm were used for the experiment. Animals were free to access drinking water and food. Animals were cared for and used in accordance with the Institutional Animal Ethics Committee (IAEC), P.G.T. Department of Zoology, RTM Nagpur University, Nagpur (Registration No.-478/01/a/CPCSEA).

For experimental induction of diabetes alloxan monohydrate (A74/3 Sigma Aldrich) was used. Diabetes was induced in 16 hrs fasted albino rats with single intraperitoneal dose of alloxan monohydrate. Alloxan injection was prepared in 0.9% normal saline. Rats with fasting blood glucose more than 220 mg/dl was considered for study. During dose standardization study it was found that 180 mg /kg intraperitoneal dose of alloxan monohydrate was suitable for diabetes induction with the 6-12 month old rats.

Experimental Animals

Healthy albino rats (9 months old) of both sexes, weighing 150-190 gm were used for the experiment. Animals were free to access drinking water and food. Animals were cared for and used in accordance with the Institutional Animal Ethics Committee (IAEC), P.G.T. Department of Zoology, RTM Nagpur University, Nagpur (Registration no.-478/01/a/CPCSEA).

For experimental induction of diabetes alloxan monohydrate **(A7413 Sigma Aldrich)** was used. Diabetes was induced in 16 hrs fasted albino rats with single intraperitoneal dose of alloxan monohydrate.

Grouping of animals

For this study rats were divided into three groups (n=6),

Group-I (NC): Kept as normal control (NC) the animals of this group was free to access drinking water and food they neither injected by alloxan nor fed on plant extract.

Group- II (DC): These group animals were injected with alloxan monohydrate (180 mg/kg bw) and kept as diabetic control. They were not fed on extract.

Group III (DC+TCE): This group was injected with alloxan monohydrate (180 mg/kg bw) and from day 2 to 30 half an hour prior to feeding, orally administrated with TCE (20 ml/kg bw) twice a day.

Histological study of pancreas

After 30 days of plant extract dosing, animals from each group were sacrifies, pancreases were dissected out and fixed in Baker's formalin. After adequate fixation tissues are transferred to 70% ethanol. Tissue is dehydrated through a series of graded ethanol baths during these the serial changes of 30%, 50% ,70% 90% and then first change to absolute, second change for absolute was given to displace the water. After completion of dehydration the tissue was then transferred to the first exposure to xylene as clearing agent after half an hour the second change of the xylene was given. The tissue was then infiltrated with two changes of paraffine wax at 60^0C. The infiltrated tissue was then embedded into wax blocks. A well trimmed paraffine block of pancreases tissue was sectioned on rocking microtome at 6μ. The ribbon of the tissue were spread over the slide with Mayer's albumin and allowed the slide for drying. After three days the slides were process for staining.

For staining with aldehyde fuschin the slides were deparaffinized with two changes of xylene and preceded for hydration with changes of 100% - water grades. After hydration slides were kept into 30% 50% and 70% ethanol for 10 minutes each. After this slides were kept in aldehyde fuschin (AF) solution for half an hour. After staining with AF three changes of 70% was given for 3 min., 2 min., 1 min. respectively. The slides were then washed in tap water for approximately 15 minutes. Stained in Mayer's Hematoxylin for 10 seconds. Again wash in running tap water for oxidation of the hematoxylin stain and observed for blue colour development. Then three dips in 50% alcohol were given this followed by three dips in eosin. The slides were then processed for quick dehydration by placing the slides into 95% ethanol for 10 minutes and then to absolute ethanol. After dehydration three changes of xylene were given. Mounting was done with Di-n-butyl phthalate in Xylene (DPX).

RESULTS AND DISCUSSION

The significant ($P<0.05$) hike in insulin secretion observed in this study from diabetic control to the normal as observed in *Tinospora Cordifolia* treated group indicates that the whole plant extract may exerts its healing effect on the islets of Langerhans. To confirm this we have performed the histological staining of pancreatic section with aldehyde fuschin.

According to Puranik (2010) the antidiabetic activity of *Tinospora Cordifolia* stem extract is not through the insulin secretion by pancreatic beta cells. They observed that *Tinospora cordifolia* treated diabetic rats did not reveal any evidence of regeneration of beta cells of islets of Langerhans. The histological section of the pancreas of the drug treated control rats showed the normal architecture of the islets of Langerhans with the granulated beta cells appearing dark. The histology of the pancreas in diabetic rats showed small and shrunken islets of Langerhans. Destruction of beta cells was observed in this section. The histology of the pancreas in *Tinospora cordifolia* treated diabetic rats showed a similar architecture to that of diabetic rats. There was no considerable change in the architecture of the islets of Langerhans after the *Tinospora cordifolia* treatment. It appears that there was no regeneration of beta cells after the *Tinospora cordifolia* treatment; therefore, it appears that the antihyperglycemic activity of *Tinospora cordifolia* is not through the insulin secretion and is independent of insulin secretion by pancreatic beta cells.

In the histological observation it was observed that the cells in the normal control animal pancrease islets was densly packed (Fig. 1). In case of alloxan treated group the cell density significantly diminish as denoted by the arrows in the (Fig. 2). The photomicrograph of the diabetic pancreatic islets clearly shows the alloxan induced damage in the form of the empty spaces.This spaces may be created due to the alloxan induced necrosis of the beta cells. As discussed previously the alloxan induced necrosis is mediated by reactive oxygen species mediated lipid peroxidation which causes bursting of plasma membrane of the cell and disturbance of osmotic balance this osmotic alteration ultimately leads the cell towards necrosis. In contrast to previous work on the *Tinospora Cordifolia* stem extract in present study we observed the healing effect of the *Tinospora Cordifolia* whole plant extract. From (Fig. 3) photomicrograph it is observed that the empty spaces in islets created by the alloxan induced necrosis is significantly decresed, this indicates that *Tinospora Cordifolia* whole plant extract stimulates the regeneration of the alloxan damaged islets.

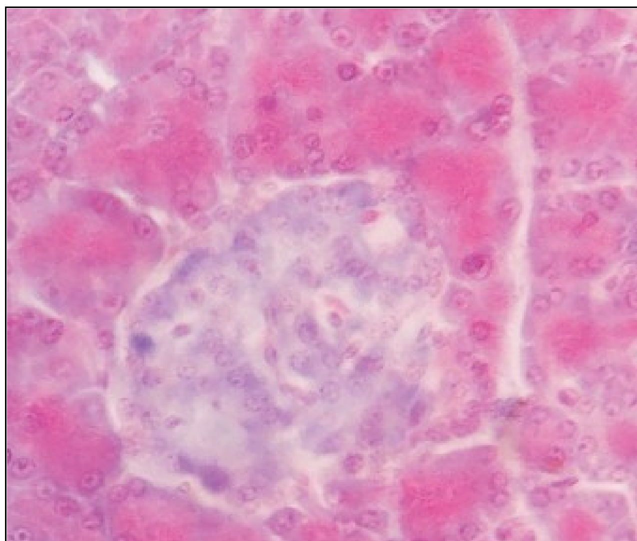


Fig . 1:

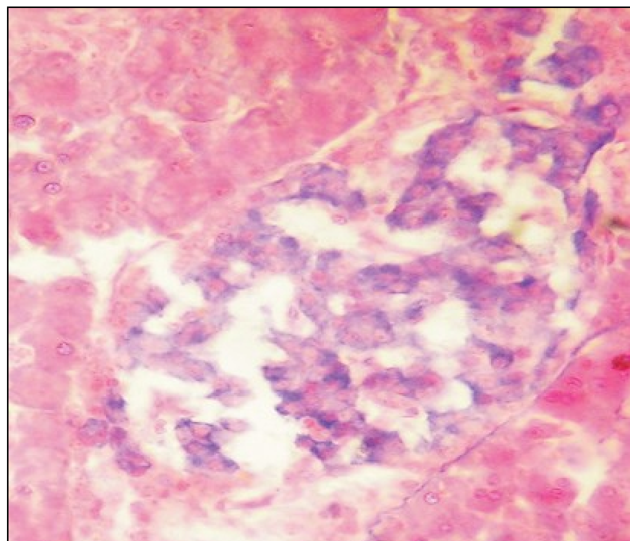


Fig. 2

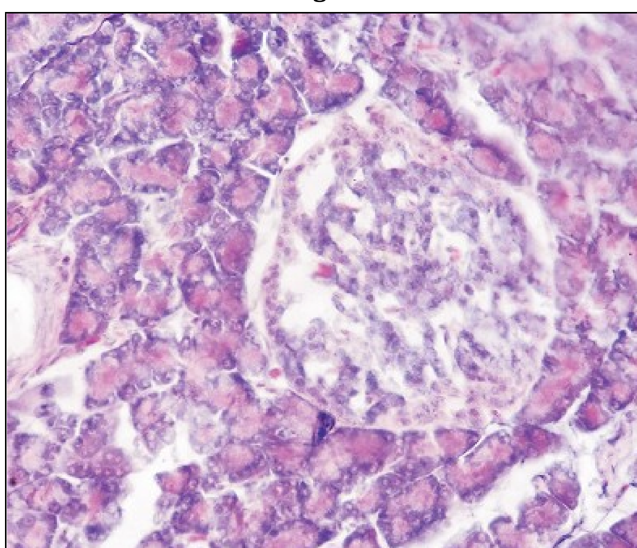


Fig 3

Fig. 1: Aldehyde fuschin stained rat pancreas photomicrograph of the normal control group. Arrow indicates healthy islets of Langerhans. X450

Fig. 2: Aldehyde fuschin stained rat pancreas photomicrograph of the diabetic control group. Arrow indicates the alloxan induced damage. X450

Fig. 3: Aldehyde fuschin stained rat pancreas photomicrograph of the diabetic control group. Arrow indicates damage remain after the *Tinospora cordifolia* extract treatment**.** X450

In nutshell, the *Tinospora cordifolia* whole plant part extract shows hypoglycemic activity in the alloxan induced diabetes. This hypoglycemic activity of the plant extract is not because of the insulin mimicking activity. *Tinospora cordifolia* whole plant part extract stimulate the pancreatic islets regeneration as observed during the histological photomicrograph. The plant extract induced regeneration of the islets responsible for the increase in the serum insulin. In addition to these activities the *Tinospora cordifolia* extract shows protective avtivity in (Reactive Oxygen Species) ROS induce damage tissues.

CONCLUSION

This study was carried out to explore the efficacy of anti-diabetic activity of *Tinospora cordifolia* whole plant extract in alloxan induced diabetic rats. The hike in insulin secretion observed in this study from diabetic control to the normal as observed in *Tinospora cordifolia* treated group indicates that the whole plant extract may exerts its healing effect on the islets of Langerhans. To confirm this we have performed the histological staining of pancreatic section with aldehyde fuschin.

The study of antidiabetic activity of the *Tinospora Cardifolia* whole plant part extract was carried out on the albino rats of both the sexes. The rats were grouped into three group i.e. normal control, diabetic control and treatment group respectively. Experimental diabetes was induced with alloxan 180 mg/kg intraperitoneal dose.

From the present study it can be concluded that the *Tinospora cordifolia* whole plant extract posses antidiabetic activity which is not by insulin mimicking activity of bioactive compound instead, it is mediated by regeneration of islets of Langerhans. Beside its antidiabetic and regeneration stimulating activity on damage pancreatic Islets the plant extract found to contain the bioactive principle which prevents reactive oxygen species mediated oxidative damage.

In the histological observation it was observed that the cells in the normal control animal pancreas Islets was densely packed (Fig. 1). In case of alloxan treated group the cell density significantly diminish as denoted by the arrows in the (Fig. 2). The photomicrograph of the diabetic pancreatic islets clearly shows the alloxan induced damage in the form of the empty spaces. This spaces may be created due to the alloxan induced necrosis of the beta cells. As discussed previously the alloxan induced necrosis is mediated by reactive oxygen species mediated lipid peroxidation which causes bursting of plasma membrane of the cell and disturbance of osmotic balance this osmotic alteration ultimately leads the cell towards necrosis. In contrast to previous work on the *Tinospora cordifolia* stem extract in present study we observed the healing effect of the *Tinospora cordifolia* whole plant extract. From (Fig. 3) photomicrograph it is observed that the empty spaces in islets created by the alloxan induced necrosis is significantly decreased, this indicates that *Tinospora cordifolia* whole plant extract stimulates the regeneration of the alloxan damaged islets.

REFERENCES

Brogard JM, Vetter T, Bickle JF (1992) Discovery of pancreatic diabetes in Strasbourg. Diabetes Metabolism; 18: 104-114.

Bliss M (2007) The discovery of insulin. University of Chicago Press, Chicago.

Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA. 1991. Encore: pancreatic extract in the treatments of diabetes mellitus: Primary report, CMAJ; 145:1281-1286.

Johnson DD (1950) Alloxan administration in the guinea pig:a study of the regenerative phase in the islets of Langerhans. Endocrinology; 47: 393-98.

Kinkar SB and Patil KG (2015) Antidiabetic Activity of *Tinospora Cordifolia* (Fam: Menispermaceae) in Alloxan Treated Albino Rats. Applied Science Journal, 1 (5): 316-319.

Kinkar SB and Patil KG (2016) Investigations on Insulin Levels and Blood Sugar Concentration in *Tinosporacordifolia* Extract Treated Albino Rats. World Journal of Zoology, 11(1): 155-158.

Kliber A, Szkudelski T, Chichlowska J (1996) Alloxan stimulation and subsequent inhibition of insulin release from in situ perfused rat pancreas. Journal of Physiology and Pharmacology; 47: 321-8.

Mythili MD, Vyas R, Akila G, Gunasekaran S (2004) Effect of Streptozotocin on the ultrastructure of rat pancreatic islets. Microsc Research Technology; 63: 274-81.

Peschke E, Ebelt H, Bromme HJ, Peschke D (2000) Classical and new diabetogens comparison of their effects on isolated rat pancreatic islets in vitro. Cell Molecular Life Science; 57: 158-64.

Ramachandran A, Jali MV, Mohan V, Snehalatha C, Viswanathan M (1988) High prevalence of diabetes in an urban population in south India. Biomedical Journal;; 297:587-90.

Sicree R, Shaw J, Zimmet P (2006) Diabetes and impared glucose tolerance. In: Gan D, editor. Diabetes atlas. International diabetes federation. 3rd ed. Belgium: International Diabetes Federation; 15-103.

Tinospora cordifolia stem bark (2006) Traditional system of medicine. New Delhi: Narosa Publishing House; 414-7.

Von Mering J, Minkowski O (1890) Diabetes mellitus nach Pankreasextirpation. Archiv For Experimentelle Pathologie And Pharmacology; 26: 371-87.

Zimmet P, Alberti KG, Shaw J (2001) Global and societal implications of the diabetes epidemic. Nature; Dec; 414 (6865): 782-787.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Estimation of Gallic acid, Rutin and Quercetin in *Portulaca quadrifida* L. – A potential wild edible plant by HPTLC method

Marathe Vishal R* and Umate Satish K

Department of Botany, N.E.S. Science College, Nanded (MS), India
*Corresponding author Email: dr.vishalmarathe@gmail.com

Manuscript details:

Received: 08.02.2016
Revised: 05.03.2016
Accepted :15.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Marathe Vishal R and Umate Satish K (2016) Estimation of Gallic acid, Rutin and Quercetin in *Portulaca quadrifida* L. – A potential wild edible plant by HPTLC method. *International J. of Life Sciences*, 4(1): 83-88.

Acknowledgement
The authors thankful to Dr. D. U. Gawai, Principal, NES Science College, Nanded for providing laboratory facilities and Dr. D. M. Jadhav, prof in-charge of Central Instrumentation Laboratory, for HPTLC facility

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

HPTLC method was developed for the quantitative estimation of gallic acid, rutin and querecetin from methanolic extract of *Portulaca quadrifida* L. a potential wild edible plant. Precoated silica gel GF_{254} used as stationary phase and mobile phase for gallic acid was Toulene: Formic acid: Ethyl acetate: Methanol [3:3:8:2, V/V/V/V] and Mobile phase for rutin and querecetin was Ethyl Acetate: Formic acid: Glacial Acetic acid: Water [10:0.5:0.5:1.3, V/V/V/V]. Detection and quantification were performed densitometrically at wavelength λ 254. The Rf values of gallic acid, rutin and quercetin are 0.41, 0.19 and 0.94 respectively. The total peak areas of the standards (gallic acid, rutin and querecetin) and the corresponding peak areas of extract were compared and the gallic acid, rutin and quercetin content were estimated to be 790.9, 2029.7 and 4326.0.

Keywords: HPTLC, Nutraceuticals, *Portulaca quadrifida* Wild Edible Plant

INTRODUCTION

Portulaca quadrifida L. is a small diffused, succulent annual herb found throughout the tropical part of India. It is said to be useful in asthma, cough, urinary discharges, inflammations, and ulcers, abdominal complaints (Kirtikar and Basu, 2001). It has been reported to possess antifungal activity (Hoffman *et al.,* 2004). Plant shows anti-diabetic properties (Khatun *et al.,* 2015). Fresh leaves of *P. quadrifida* slightly wormed and applied topically in joint swelling (Abbasi *et al.,* 2013), It shows depressant effect of ethanolic extract on CNS (Syed *et al.,* 2010). The leaves and tender shoots of plant are cooked as vegetables by tribal and local peoples in Maharashtra and rest part of India (Reddy 2012; Naik 1998; Raphel and Britto 2015).

Wild edible plants play a very important role in the diet of tribal communities. They are major source of food for tribals of forest areas. Edible parts of wild plants are promising gift of nature to mankind, these are not only delicious and refreshing but also the chief source of vitamins, minerals, proteins and other nutrients.

'Nutraceutical' the term coined in 1979 by Stephan De Felice. It is designed as a food or parts of food that provide medical or health benefits, including the prevention and treatment of disease (De Felice 1992). Nutraceutical may range from isolated nutrients, dietary supplements, herbal products and processed products. Nutraceutical play important role in physiological benefits or provide protection against the diseases (Rajsekaran *et al.,* 2008).

The major nutraceutical ingredients in plant are phenolic compounds mainly Flavonoids (Tapas *et al.,* 2008). Gallic acid (3, 4, 5,-tryhydroxybenzoic acid) is naturally occurring polyphenolic compounds posses astringent, antioxidative, antimicrobial activity (Prince *et al.,* 2009, Urizzi *et al.,* 1999, Verma *et al.,* 2013). They also constitute an unavoidable component of the diet. Rutin and quercetin are phenolic compounds exhibit antiulcer, anti-inflammatory, antioxidant, antimicrobial, antiallergic activity (Agnes *et al.,* 2008, Maalik *et al.,* 2014, Gupta *et al.,* 2014, Singh and Bilashini 2015). They have shown regulatory activity of hormones such as transport, metabolism and action of thyroid harmones (Ashok *et al.,*2010).

High performance thin layer chromatography (HPTLC) has emerged as an useful analytical method for qualitative and quantitative estimation of chemical constituents present in plant materials (Sethi, 1996). Present study deals with estimation of important nutraceuticals and antioxidants like gallic acid, quercetin and rutin in *Portulaca quadrifida* L. by HPTLC method.

MATERIAL AND METHODS

Preparation of extract

The aerial part of wild edible plant *Portulaca quadrifida* L. were collected from different parts of Nanded district. The plant was identified and authenticated. Edible part of plants were dried and made into coarse powder and stored in sealed container. Powder then extracted with methanol by Soxhlet apparatus and concentrated.

Reagents and other materials

Gallic acid, rutin and quercetin [Sigma Aldrich] toluene, formic acid, ethyl acetate, methanol, glacial acetic acid, [all reagents of analytical grade, E-Merck] and silica gel F_{254} TLC aluminium plates [E-Merck].

Preparation of standard and sample solutions

Gallic acid, rutin and quercetin 10mg were accurately weighed into10mL volumetric flask dissolved in 10 mL of methanol [1mg/mL]. The 100 mg of extract was dissolved in methanol [10mL] then solution was filtered through whattman filter paper No. 42.

Development of HPTLC Technique

The sample were spotted in the form of bands with micro litre syringe on pre-coated silica gel plates F_{254} [10 cm x 10 cm with 0.2 mm thickness] using CAMAG Linomat 5 applicator automatic sample spotter of band width 6mm. The plates were developed in a solvent system in CAMAG glass twin through chamber previously saturated with the solvent for 30 min. The distance was 8 cm subsequent to the scanning, TLC plates were air dried and scanning was performed on a CAMAG TLC Scanner in absorbance at 254 nm and operated with win CATS Planar chromatography Manager.

Gallic acid estimation in P. qudrifida L.

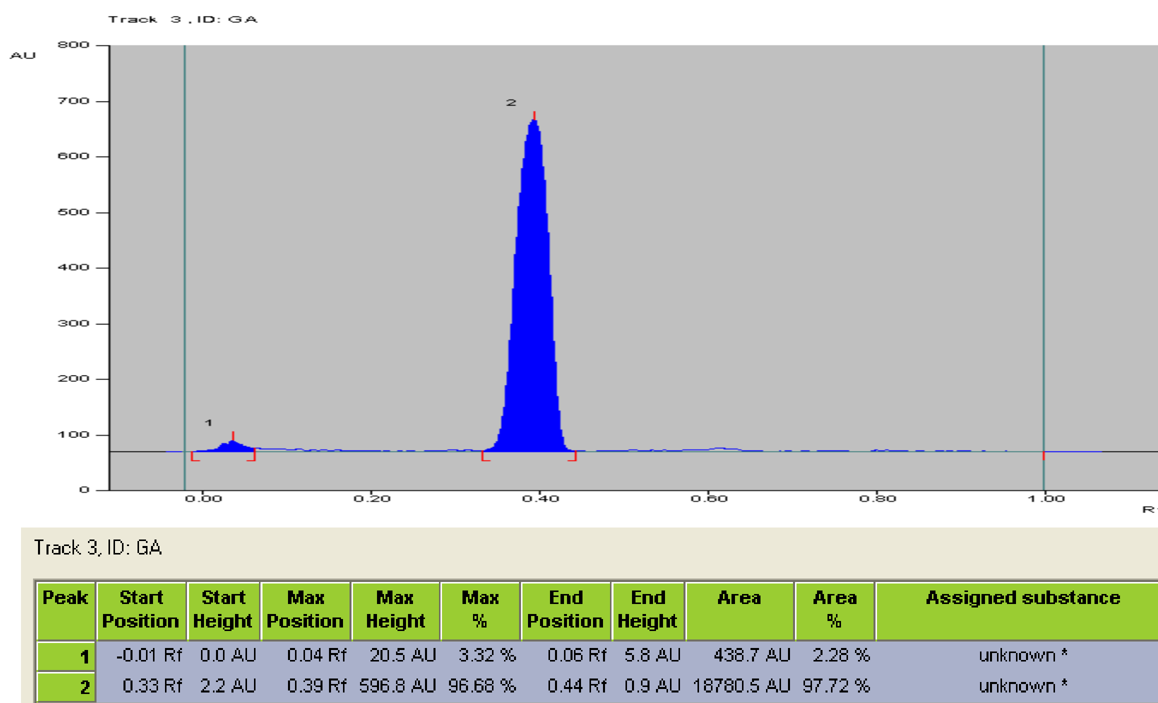
Stationary phase- silica gel F_{254} plates, Mobile phase- Toulene: Ethyl acetate: Formic acid: Methanol [3:3:8:2 v/v/v/v/v], standard Gallic acid 1 mg/ml[5 µl], sample Methanol extract 10mg/ml[10 µl], Migration distance 80 mm, scanning wavelength 254 mm, Mode of scanning Absorption [deuterium].

Rutin and Quercetin estimation in P. quadrifida L.

Stationary phase- silica gel F_{254} plates, Mobile phase- Ethyl acetate: Formic acid: Glacial acetic acid: Water [10:0.5:0.5:1.3 v/v/v/v/v], standard Rutin and Quercetin 1 mg/ml [5 µl], sample Methanol extract 10mg/ml [10 µl], Migration distance 80 mm, scanning wavelength 254 mm, Mode of scanning Absorption [deuterium].

RESULTS AND DISCUSSION

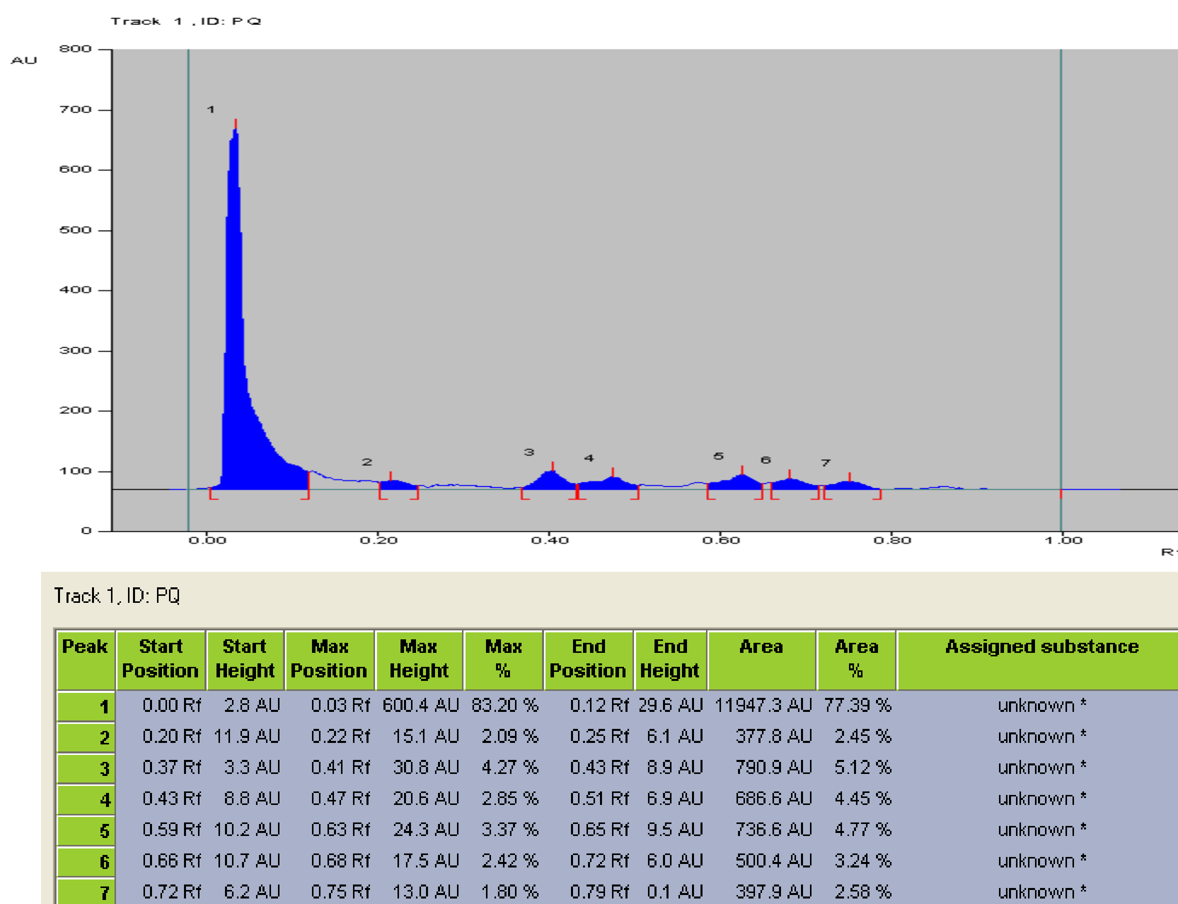
The Rf value of standard gallic acid was found to be 0.39 and the peak area 18780.5 [fig. 1]. Methanolic extract of *P. quadrifida* L. showed seven peaks [fig.2], the third peak Rf value 0.41was coinciding with standard Rf values and its peak area was 790.9. The Rf values of standard rutin and quercetin was found to be 0.14 and 0.92 and peak area was 16522.0 and 8088.1 respectively [fig.3, 4]. Methanolic extract of plant showed eight peaks the third and seventh peaks of Rf values 0.19 and 0.94 was coinciding with standard Rf value, peak area was found 2029.7 for rutin and 4326.0 for quercetin [fig.5].



Track 3, ID: GA

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.01 Rf	0.0 AU	0.04 Rf	20.5 AU	3.32 %	0.06 Rf	5.8 AU	438.7 AU	2.28 %	unknown *
2	0.33 Rf	2.2 AU	0.39 Rf	596.8 AU	96.68 %	0.44 Rf	0.9 AU	18780.5 AU	97.72 %	unknown *

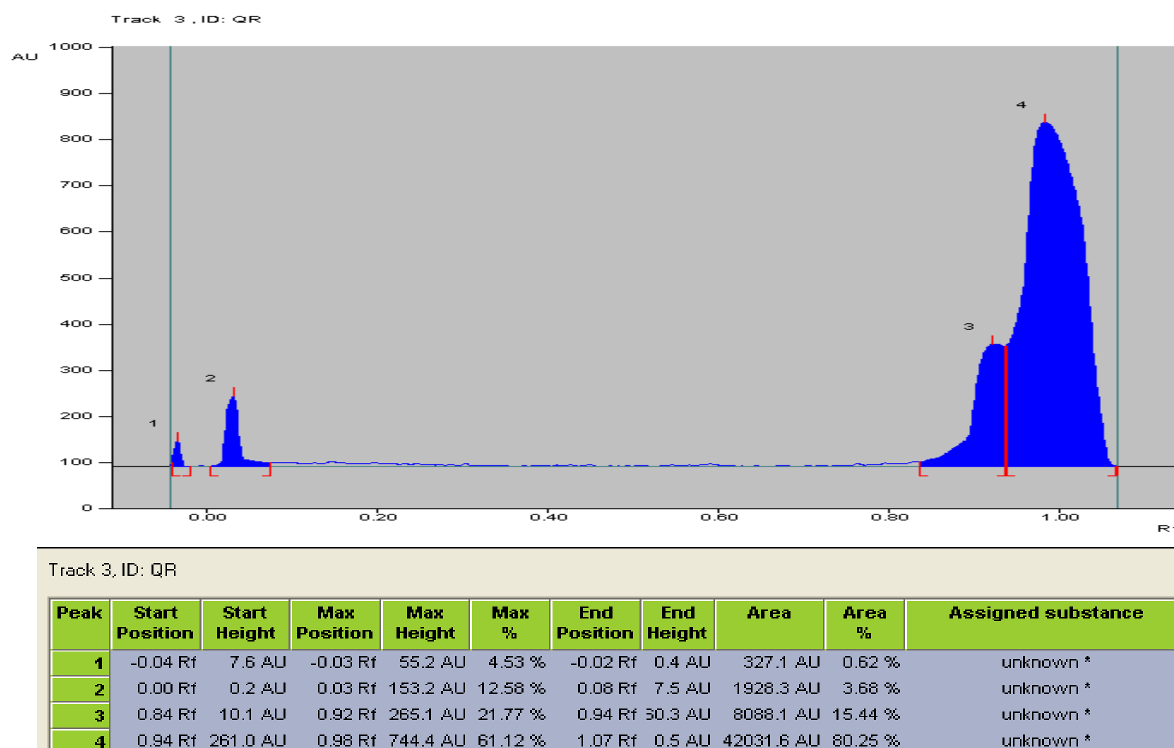
Figure 1: HPTLC Profile for Gallic acid standard



Track 1, ID: PQ

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.00 Rf	2.8 AU	0.03 Rf	600.4 AU	83.20 %	0.12 Rf	29.6 AU	11947.3 AU	77.39 %	unknown *
2	0.20 Rf	11.9 AU	0.22 Rf	15.1 AU	2.09 %	0.25 Rf	6.1 AU	377.8 AU	2.45 %	unknown *
3	0.37 Rf	3.3 AU	0.41 Rf	30.8 AU	4.27 %	0.43 Rf	8.9 AU	790.9 AU	5.12 %	unknown *
4	0.43 Rf	8.8 AU	0.47 Rf	20.6 AU	2.85 %	0.51 Rf	6.9 AU	686.6 AU	4.45 %	unknown *
5	0.59 Rf	10.2 AU	0.63 Rf	24.3 AU	3.37 %	0.65 Rf	9.5 AU	736.6 AU	4.77 %	unknown *
6	0.66 Rf	10.7 AU	0.68 Rf	17.5 AU	2.42 %	0.72 Rf	6.0 AU	500.4 AU	3.24 %	unknown *
7	0.72 Rf	6.2 AU	0.75 Rf	13.0 AU	1.80 %	0.79 Rf	0.1 AU	397.9 AU	2.58 %	unknown *

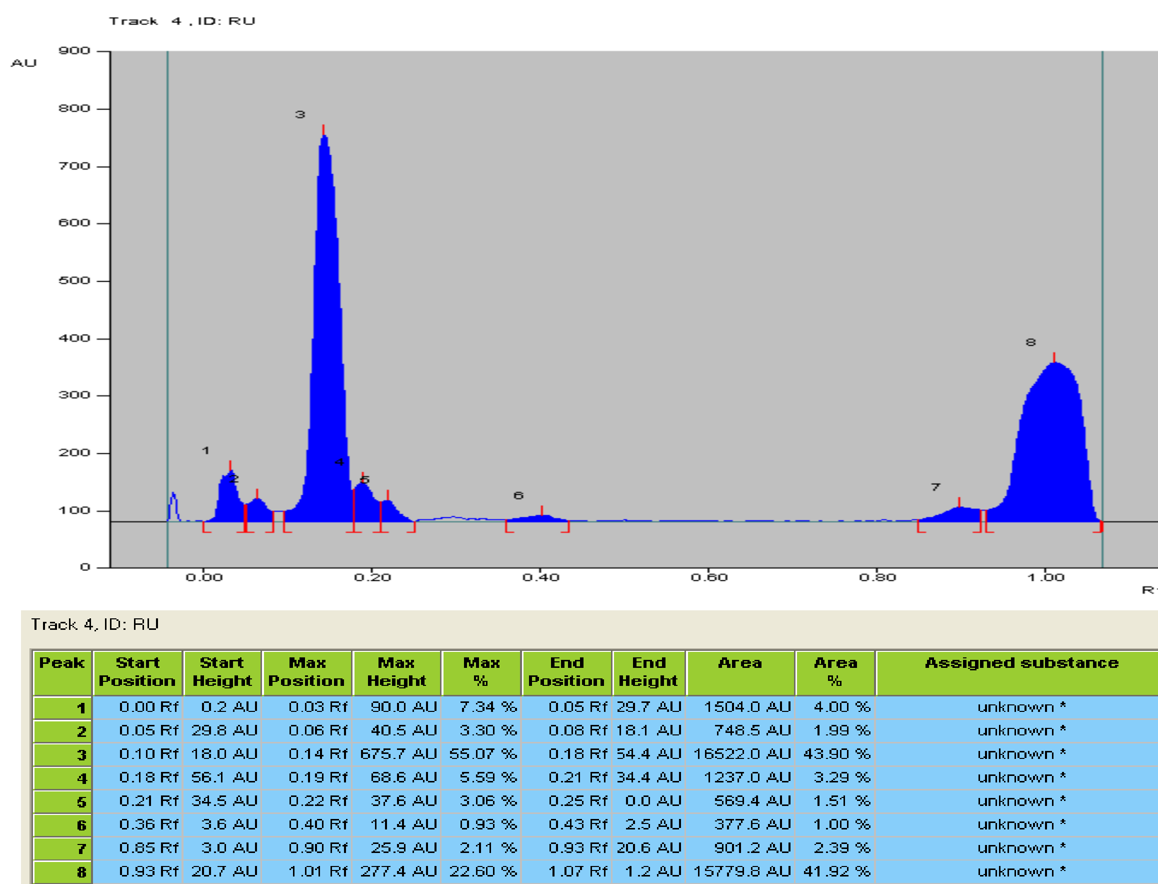
Figure 2: HPTLC Profile for MeOH Extract of *P. quadrifida* L.



Track 3, ID: QR

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	7.6 AU	-0.03 Rf	55.2 AU	4.53 %	-0.02 Rf	0.4 AU	327.1 AU	0.62 %	unknown *
2	0.00 Rf	0.2 AU	0.03 Rf	153.2 AU	12.58 %	0.08 Rf	7.5 AU	1928.3 AU	3.68 %	unknown *
3	0.84 Rf	10.1 AU	0.92 Rf	265.1 AU	21.77 %	0.94 Rf	30.3 AU	8088.1 AU	15.44 %	unknown *
4	0.94 Rf	261.0 AU	0.98 Rf	744.4 AU	61.12 %	1.07 Rf	0.5 AU	42031.6 AU	80.25 %	unknown *

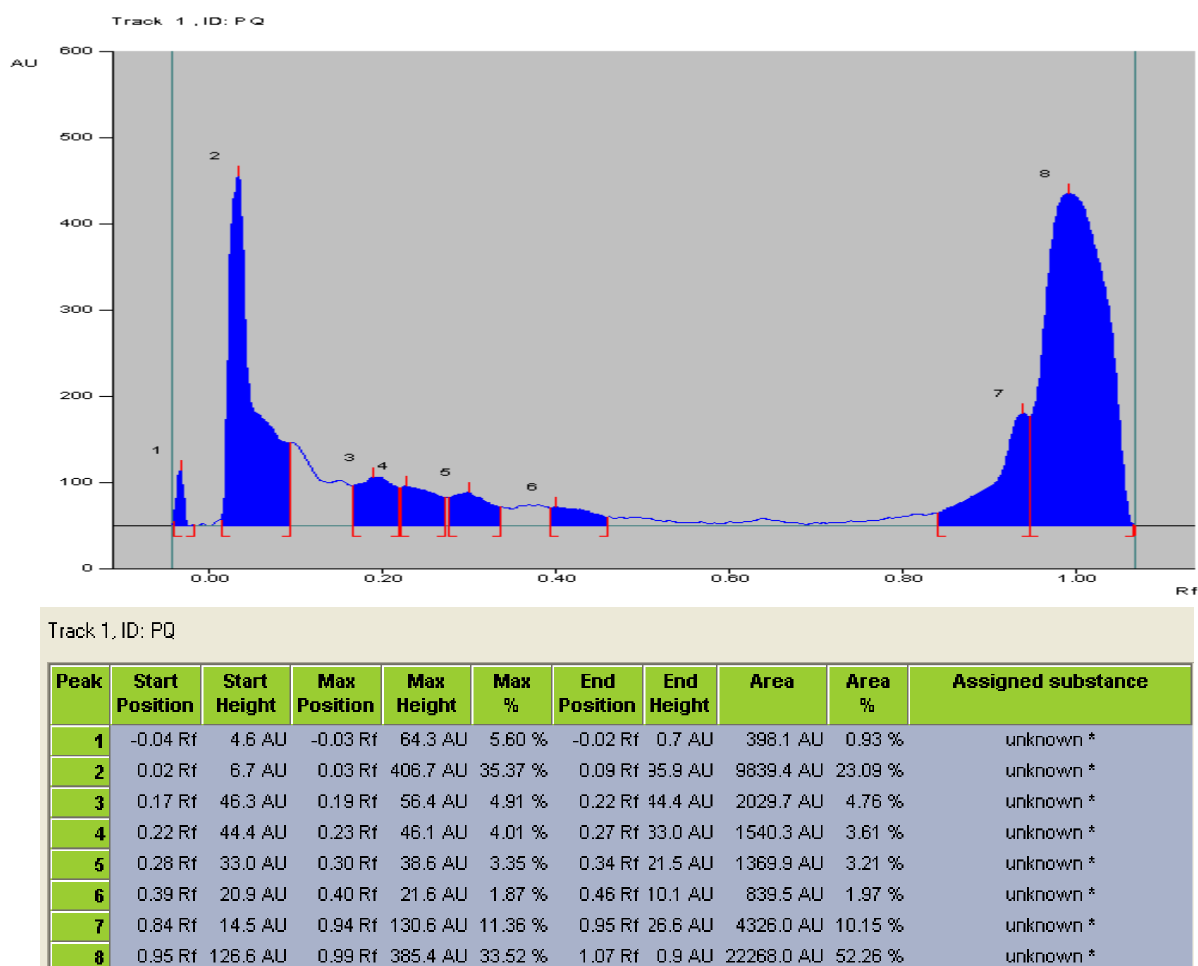
Figure 3: HPTLC Profile for Quercetin standard



Track 4, ID: RU

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.00 Rf	0.2 AU	0.03 Rf	90.0 AU	7.34 %	0.05 Rf	29.7 AU	1504.0 AU	4.00 %	unknown *
2	0.05 Rf	29.8 AU	0.06 Rf	40.5 AU	3.30 %	0.08 Rf	18.1 AU	748.5 AU	1.99 %	unknown *
3	0.10 Rf	18.0 AU	0.14 Rf	675.7 AU	55.07 %	0.18 Rf	54.4 AU	16522.0 AU	43.90 %	unknown *
4	0.18 Rf	56.1 AU	0.19 Rf	68.6 AU	5.59 %	0.21 Rf	34.4 AU	1237.0 AU	3.29 %	unknown *
5	0.21 Rf	34.5 AU	0.22 Rf	37.6 AU	3.06 %	0.25 Rf	0.0 AU	569.4 AU	1.51 %	unknown *
6	0.36 Rf	3.6 AU	0.40 Rf	11.4 AU	0.93 %	0.43 Rf	2.5 AU	377.6 AU	1.00 %	unknown *
7	0.85 Rf	3.0 AU	0.90 Rf	25.9 AU	2.11 %	0.93 Rf	20.6 AU	901.2 AU	2.39 %	unknown *
8	0.93 Rf	20.7 AU	1.01 Rf	277.4 AU	22.60 %	1.07 Rf	1.2 AU	15779.8 AU	41.92 %	unknown *

Figure 4: HPTLC Profile for Rutin standard



Track 1, ID: PQ

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	4.6 AU	-0.03 Rf	64.3 AU	5.60 %	-0.02 Rf	0.7 AU	398.1 AU	0.93 %	unknown *
2	0.02 Rf	6.7 AU	0.03 Rf	406.7 AU	35.37 %	0.09 Rf	35.9 AU	9839.4 AU	23.09 %	unknown *
3	0.17 Rf	46.3 AU	0.19 Rf	56.4 AU	4.91 %	0.22 Rf	44.4 AU	2029.7 AU	4.76 %	unknown *
4	0.22 Rf	44.4 AU	0.23 Rf	46.1 AU	4.01 %	0.27 Rf	33.0 AU	1540.3 AU	3.61 %	unknown *
5	0.28 Rf	33.0 AU	0.30 Rf	38.6 AU	3.35 %	0.34 Rf	21.5 AU	1369.9 AU	3.21 %	unknown *
6	0.39 Rf	20.9 AU	0.40 Rf	21.6 AU	1.87 %	0.46 Rf	10.1 AU	839.5 AU	1.97 %	unknown *
7	0.84 Rf	14.5 AU	0.94 Rf	130.6 AU	11.36 %	0.95 Rf	26.6 AU	4326.0 AU	10.15 %	unknown *
8	0.95 Rf	126.6 AU	0.99 Rf	385.4 AU	33.52 %	1.07 Rf	0.9 AU	22268.0 AU	52.26 %	unknown *

Figure 5: HPTLC Profile for MeOH Extract of *P. quadrifida* L.

CONCLUSION

HPTLC Analysis of *P. quadrifida* shows good concentration of gallic acid, quercetin and rutin which proves its antioxidant nature. The results of present study support its edible nature and it could be potential source of nutraceutical and natural antioxidant.

REFERENCES

Abbasi AR, Khan MA, Shah MH, Shah MM, Parvez A and Ahemad M (2013) Ethanobotanical appraisal and cultural values of medicinally important wild vegetables of lesser Himalayas-Pakistan.: *J. Of ethnobotany and ethnomedicine* 9: 66.

Agnes WB, Guido RMM, Haenen, AaltBast (2008) Health Effects of Quercetin: From Antioxidant to Nutraceutical: *Euro. J. Of Pharmaco.* 585, 325-337.

Ashok Kumar, Lakshman K, Jayaveera KN, Mani Tripathi SN and Satish KV (2010) Estimation of Gallic acid, Quercetin and Rutin in *Terminalia chebula* by HPTLC. *Jordan J. Of Pharmaceutical Sci.*, Vol.3, No.1, 63-67.

De Felice SL (1992) Nutraceuticals: Opportunities in an emerging market., *Scrip. Mag.*: 9.

Gupta N, Chauhan RS, Pradhan JK (2014) Rutin: A Bioactive Flavonoids; Handbook of Medicinal Plants and Their Bioactive Compounds, 51-57.

Hoffman BR, DelasAtlas H, Bianco K, Wiederhold N, Lewis RE, Williams L (2004) Screening of antibacterial and antifungal activities of ten medicinal plants from Ghana. *Pharmaceutical Biolog*, 42 (1): 13-17.

Khatun M, Shariful H, Md. Abdul H, Sheikh NI (2015) Medicinal value of some edible leafy vegetables from Bangladesh. *World Journal of Pharmaceutical Research*. 4 (2): 139-151.

Kirtikar VR and Basu BD, 2001. Indian Medicinal plants, IInd edition, Vol. 6, Prakashan publications, Mumbai; 1447.

Maalik A, Khan FA, Amara Mumtaz, Adeem Mehmood, Saira Azhar, Muhammad A, Sabiha Karim, YasirAltaf and Imran Tariq (2014) Pharmacological applications of Quercetin and its derivatives: A Short Review. *Tropical J. of Pharma. Res.* 13(9):1561-1566.

Naik VN (1998) The Flora of Marathwada Vol. I and Vol. II, Amrut Prakashan, Aurangabad, M.S. (India).

Prince PSM, Priscilla H, Devika PT (2009) Gallic acid prevents lysosomal damage in isoproterenol induced cardiotoxicity in Wistar rats. *Eur. J. Pharmacol*. 615(1)139-143.

Rajasekaran A, Sivagnanam G, Xavier R (2008) Nutraceuticals as therapeutic agents: A Review, *Research Journal pharm. and tech*, 1(4), 328-340.

Raphael RM and Britto JS (2015) Medicinal properties of edible weeds of crop fields and wild edible plants eaten by Oraon tribals of Letehar District, Jharkhand, *International Journal of lifesci. And pharma. Re.* 5(2): 9-20.

Reddy BM (2012) Wild Edible Plants of Chandrapur district, Maharashtra, India, *Indian Journal of Natural Products and resources*, 3(1):110-117.

Sethi PD (1996) HPTLC, CBS Publishers and distributors I[st] edition, New Delhi, 39.

Singh S, Mayanglambam BD (2015) Vegetables as a potential source of Nutraceuticals and phytochemicals: A Review; *Int. J. Of Medicin* and *Pharmace. Sci.*; 5(2), 1-14.

Syed KM, Ahmed LD and Paramjyothi S (2010) Neuropharmacological effects of ethanolic extract of *Portulaca quadrifolia* Linn. In mice, *Int. J. of pharma Res.* 2(2): 1386-1390.

Tapas AR, Sakarkar DM, Kakde RB (2008) Flavonoids as Nutraceuticals: A Review; *Tropical Journal of pharmaceutical Research*, **7**(3): 1089-1099.

Urizzi P, Monje MC, Souchard JP, Abella A, Chalas J, Lindenbaum A, Vergnes L, Labidalle S, Nepveu F (1999) Antioxidant activity of phenolic acid and esters present in red wine on human low density lipoproteins. *J Chem. Phys*. 96:110-115.

Verma S, Singh A, Mishra A (2013) Gallic acid: Molecular rival of cancer, *Environ. Toxicol. Pharma J.*, 35(3): 473-485.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

GIS- based morphometric analysis of major watersheds of Tehran- Karaj, Central of Iran

Sajjad Fazel Tavassol* and Gopalakrishna GS

Department of Studies in Earth Science, Manasagangotri, University of Mysore, Mysore 570 006, India.
*Corresponding author E-mail : s.fazeltavasol@yahoo.com

Manuscript details:

Received: 06.12.2015
Revised: 17.012.2015
Revise received: 04.01.2016
Accepted: 16.02.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Sajjad Fazel Tavassol and Gopalakrishna GS (2016) GIS- based morphometric analysis of major watersheds of Tehran- Karaj, Central of Iran. *International J. of Life Sciences*, 4(1): 89-96.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

The study area, Tehran-Karaj plain is situated in central part of Iran, lies between latitudes 36°10′ to 35°04′ N and longitudes 50°38′, to 51°45′ E covering an area of 4762sq km comprising of 4 sub-watersheds (A,B,C and D). Morphometric analysis is important in any hydrological investigation and it is inevitable in development and management of drainage basin. The present study involves the Geographic Information System (GIS) analysis techniques to evaluate and compare linear relief and aerial morphometric of the 4 subwater sheds. The drainage network shows that the terrain exhibits dendritic to sub-dendritic drainage pattern. Stream orders ranges from fourth to fifth order. Drainage density varies between 0.21 and 0.4 km/km^2 and has very coarse to coarse drainage texture. The relief ratio is ranging from 0.004 to0.017. The mean bifurcation ratio varies from 2.37 to 3.77 and falls under normal basin category. The elongation ratio shows that 'C' watershed possesses circular shape while remaining sub-watersheds mark elongated pattern.

Keywords: Morphometric analysis, GIS and Remote sensing , Tehran – karaj water shed, sub water shed

INTRODUCTION

The assessment of hydrological characteristics of a drainage basin is a mandate for any basin management scheme. It involves a detailed morphometric analysis, which includes basin size, shape, slope of drainage area, drainage density, size and length of the tributaries, etc. Drainage basins are the fundamental units of the fluvial landscape and a great amount of research has focused on their geometric characteristics, which include the topology of the stream networks and quantitative description of drainage texture, pattern, shape, and relief characteristics (Abrahams 1984; Huggett and Cheesman 2002).

The correlation between physiographic characteristics of drainage basin to various hydrologic phenomena has been reported by Rastogi and Sharma (1976). In various articles, morphometric analyses were used for basin characterization (Miller 1953; Boulton 1968; Gregory and Walling

1973; Gardiner 1975; Costa 1987; Topaloglu 2002; Moussa 2003; Mesa 2006; Angillieri 2008; Magesh et al. 2011; Magesh et al. 2012, John Wilson et al. 2012). Delineation of drainage networks within a basin orsub-basinor watershed can be achieved using traditional methods such as field observations and topographic maps or alternatively with advanced methods using remote sensing and GIS-(Verstappen 1983; Rinaldo et al. 1998; Macka 2001; Maidment 2002; Ozdemir and Bird 2009). The major drawback in traditional approach is its tedious effort to examine all stream networks from field observations due to their extent over a vast area. On the other hand, extraction of drainage networks from digital elevation models (DEMs) is quite handy as it assumes that water will flow from higher to lower elevation using the steepest descent. But it requires systematic method to get the results. Unifying these steps in GIS model builder using appropriate geo-processing tools will produce an automated stream extraction model with supporting thematic layers such as aspect, slope, relief, and drainage density. The output of this model can be used for further morphometric analysis.

Study Area

The study area, Tehran-Karaj plain is situated in central part of Iran, lies between latitudes 36°10′ to 35°04′ N and longitudes 50°38′, to 51°45′ E covering an area of 4762sq km. The average height of the region is 4326 m above MSL (Fig.1).

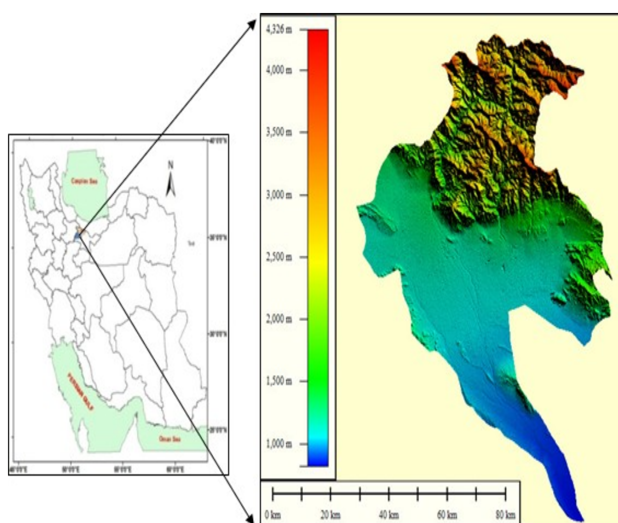


Fig. 1: Location map of the study area.

MATERIALS AND METHODS

In the present study, the map showing drainage details have been prepared from digital data of MR SIDE photo. These satellite images have been geo-referenced and merged using Image Processing software ERDAS IMAGINE9.1 and Global Mapper 15. Thus the merged data (Fig. 2) were used in the study area.

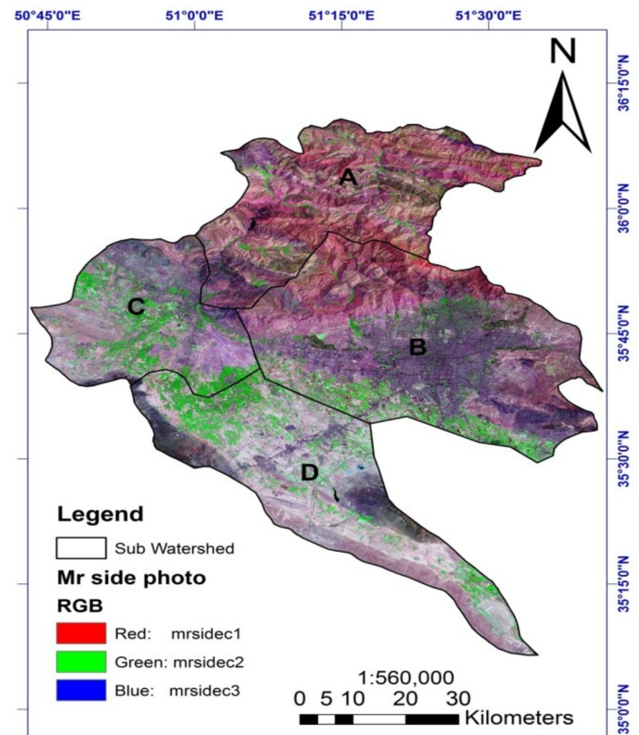


Fig. 2. Sub-watersheds on satellite image of the study area use Mrside photo

The drainages have been delineated using merged satellite data of on 1:50,000 scale and SOl toposheets have been used as a reference. The morphometric parameters considered for the analysis are summarized in detail in Table 1. GIS software like ArcInfo (V 10.2.2) have been used for digitization and computational purpose and also for output generation.

RESULTS AND DISCUSSION

The present study emphasizes the use of satellite remote sensing and Arc GIS for morphometric analysis and the results are discussed below.

Watershed Delineation

Watershed is a natural hydrological entity from which surface runoff flows to a defined drain, channel, stream or river at particular point. The entire area has been divided into 4 watersheds (Table 2). The watersheds have been named as based on the Alphabetical listing at the outlet. (Fig. 3).

Delineation of Drainage Network

The present drainage network in the 4 watersheds delineated using merged satellite data (Fig. 2)

Morphometric Analysis

Morphometric is the measurement and mathematical analysis of the configuration of the Earth's surface, shape and dimensions of its landforms (Clarke, 1966). This analysis can be achieved through measurement of linear, aerial and relief aspects of the basin and slope contributions (Nag and Chakraborty, 2003). In the present study, the morphometric analysis for the parameters namely stream order, stream length, bifurcation ratio, stream length ratio, basin length, drainage density, stream frequency, elongation ratio, circularity ratio, form factor, relief ratio, etc., has been carried out using the mathematical formulae given in Table 1 and the results are summarized in Table2.

Table 1: Methodology adopted for computations of morphometric parameters.

S.N.	Mophometric Parameters	Formula	Reference
1	Stream Order	Hierarchial rank	Strahler (1964)
2	Stream Length (Lu)	Length of the Stream	Horton (1945)
3	Mean Stream Length (Lsm)	Lsm = Lu t Nu Where, Lsm = Mean Stream Length Lu = Total stream length of order 'u' Nu = Total no. of stream segments of order 'u'	Strahler (1964)
4	Stream Length Ratio (RL)	RL=Lu/Lu-I Where, RL = Stream Length Ratio Lu = The total stream length of the order 'u' Lu - I = The total stream length of its next lower order	Horton (1945)
5	Bifurcation Ratio (Rb)	Rb= Nu/Nu+ 1 Where, Rb - Bifurcation Ratio Nu = Total no. of stream segments of order 'u' Nu + 1 = Number of segments of the next higher order	Schumn (1956)
6	Mean bifurcation ratio (Rbm)	Rbm = Average of bifurcation ratios of all orders	Strahler (1957)
7	Relief Ratio (Rh)	Rh = H / Lb Where, Rh = Relief Ratio H =Total relief (Relative relief)of the basin in Kilometers Lb = Basin length	Schumm(1956)
8	Drainage density (Dd)	D=Lu/A Where, D = Drainage Density Lu = Total stream length of all orders A = Area of the Basin (km'-)	Horton (1932)
9	Stream Frequency (Fs)	Fs=Nu/A Where, Fs = Stream Frequency Nu = Total no. of streams of all orders A = Area of the Basin (km-')	Horton (1932)
10	Drainage Texture (Rt)	Rt=Nu/P Where, Rt = Drainage Texture Nu = Total no. of streams of all orders P = Perimeter (km)	Horton (1945)
11	Form Factor (Rf)	Rf = A / Lb 2 Where, Rf = Form Factor A = Area of the Basin (km-') Lb 2 = Square of Basin length	Horton (1932)
12	Circularity Ratio (Rc)	Re=4* Pi*A/P 2 Where, Rc = Circularity Ratio Pi = 'Pi' value i.e., 3.14 A = Area of the Basin (kin 2) p2 = Square of the Perimeter (Km)	Miller (1953)
13	Elongation Ratio (Re)	Re **= 2 v (A / Pi) /** Lb Where, Re = Elongation Ratio A = Area of the Basin (km'-) Pi = 'Pi' value i.e., 3.14 Lb = Basin length	Schumn (1956)
14	Length of Overland flow (Lg)	Lg=l/D*2 Where, Lg = Length of Overland flow D = Drainage Density	Horton (1945)

Table 2: Results of morphometric analysis of 4 watersheds

SWSD No.	Stream Order	Basin Area (km2)	Stream order (Sμ)					Stream length (Lμ) (kms)					Perimeter (P) (km)	Basin length (km)
			I	II	III	IV	V	I	II	III	IV	V		
A	IV	1096.8	43	10	2	1	-	275	59.9	15.2	49.97	-	186.61	57.2
B	IV	1687.8	35	10	2	1	-	190	97.9	65.15	1.17	-	185.29	45.8
C	V	788.37	38	10	3	2	1	170.4	83.92	25.93	17.8	18.35	126.11	25.3
D	V	1189.2	11	2	1	1	1	81.51	8.4	55.55	2.1	81.96	201.45	76.1

SWSD No	Mean Stream Length in Km (Lsm)					Stream Length Ratio (RL)				Total Relief (M)	Relief Ratio (Rh)	Elongation Ratio (Re)	Texture Ratio (Rt)
	I	II	III	IV	V	II /I	III /II	IV /III	V /IV				
A	6.4	5.99	7.6	49.97	-	0.22	0.25	3.29	-	1000	0.017	0.65	0.98
B	5.43	9.79	32.57	1.17	-	0.52	0.67	0.018	-	350	0.008	1.01	1.05
C	4.48	8.39	8.64	8.9	18.35	0.49	0.31	0.69	1.04	154	0.006	1.25	2.13
D	7.41	4.2	27.7	2.1	81.96	0.1	6.61	0.038	39.02	299	0.004	0.51	0.21

SWSD No	**Bifurcation Ratio (Rb)**				Mean Bifurcation Ratio (Rbm)	Drainage Density (D) (km/km 2)	Stream Frequency (Fs)	Form Factor (Rf)	Circularity Ratio (Rc)	Length of Overland Flow (Lg)
	I /II	**II/ III**	**III /IV**	**IV /V**						
A	**4.3**	**5**	**2**	**-**	**3.77**	**0.36**	**0.051**	**0.34**	**0.4**	**1.39**
B	**3.5**	**5**	**2**	**-**	**3.5**	**0.21**	**0.028**	**0.8**	**0.62**	**2.38**
C	**3.8**	**3.33**	**1.5**	**2**	**2.66**	**0.4**	**0.068**	**1.24**	**0.62**	**1.25**
D	**5.5**	**2**	**1**	**1**	**2.37**	**0.19**	**0.014**	**0.2**	**0.37**	**1.35**

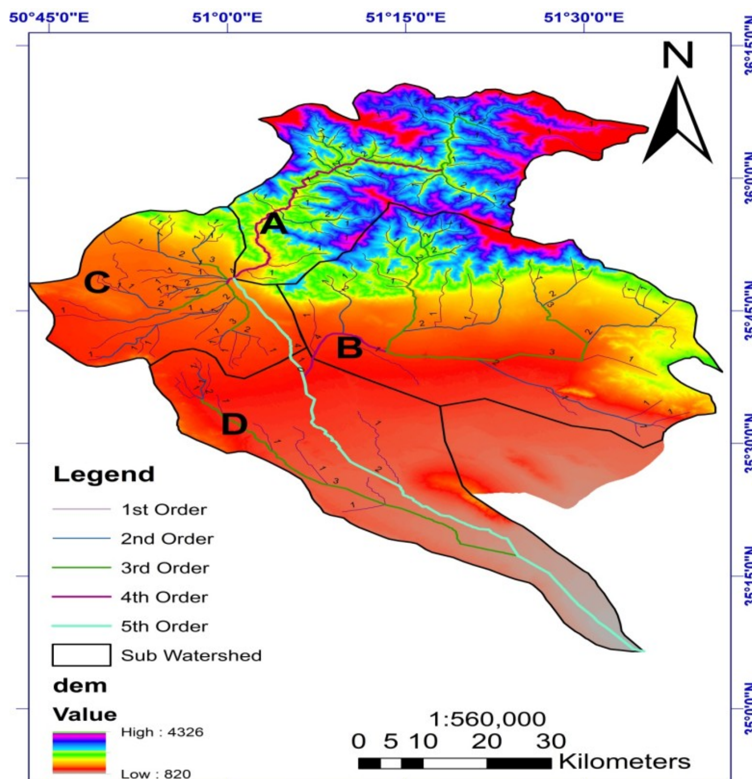


Fig. 3. Sub-watersheds showing stream orders.

Linear Aspect

Stream order, stream length, mean stream length, stream length ratio and bifurcation ratio are linear aspects, that were determined and results have beengiven in Table 2.

Stream Order:

The designation of stream orders is the first step in drainage basin analysis and is based on a hierarchic ranking of streams. In the present study, ranking of streams has been carried out based on the method proposed by Strahler (1964) (Table 1). The order wise stream numbers, area and stream lengths of the 4 watersheds are given in Table2. Out of these watersheds, 'A' and 'B' are of fourth order, while remaining sub watersheds namely 'C' and 'D' are of fifth order. It is observed from the Table 2 that the maximum frequency is in case of first order streams. It is also noticed that there is a decrease in stream frequency as the stream order increases.

Stream length:

The number of streams of various orders in a watershed are counted and their lengths from mouth to drainage divide are measured (Table 2) with the help of GIS software. The stream length (Lu) has been computed based on the law proposed by Horton (1945) for all the 4 watersheds (Table 1). Generally, the total length of stream segments is maximum in first order streams and decreases as the stream order increases. (Table 2). This change may indicate that the flowing of streams from high altitude, with lithological variation and moderately steep slopes (Singh and Singh, 1997).

Mean stream length

According to Strahler (1964), the mean stream length is a characteristic property related to the drainage network and its associated surfaces. The mean stream length (Lsm) has been calculated by dividing the total stream length of order 'u' and number of streams of segment of order 'u' (Table1). It is noted from the results (Table 2) that Lsm varies from 1.17 to 81.96 and Lsm of any given order is greater than that of the lower order and less than that of its next higher order in 'C' and 'D'-watersheds..

Stream Length Ratio

Stream length ratio (RL) may be defined as the ratio of the mean length of the one order to the next lower order of stream segment (Table 1). Horton's law (1945) of stream length states that mean stream length segments of each of the successive orders of a basin tends to approximate a direct geometric series with streams length increasing towards higher order of streams. The RL between streams of different order in the study area reveals that there is a variation in RL in each sub-watershed (Table2). This variation might be due to changes in slope-and topography. A and B sub-watersheds show an increasing trend in the length ratio from lower order to higher order indicating their mature geomorphic stage. Whereas in remaining sub-watersheds, there is a change from one order to another order indicating their late youth stage of geomorphic development (Singh and Singh, 1997).

Bifurcation Ratio

The term bifurcation ratio (Rb) may be defined as the ratio of the number of the stream segments of given order to the number of segments of the next higher order (Schumn, 1956) (Table 1). Horton (1945) considered the bifurcation ratio as an index of relief and dissections. Strahler (1957) demonstrated that bifurcation ratio shows a small range of variation for different regions or for different environment except where the powerful geological control dominates. It is observed from the Table 2, the Rb is not same from one order to its next order. These irregularities are dependent upon the geological and lithological development of the drainage basin (Strahler, 1964). The lower values of Rb are characteristics of the sub-watersheds which have suffered less structural disturbances (Strahler, 1964) and the drainage patterns has not been distorted because of the structural disturbances (Nag, 1998). In the present study, the higher values of Rb indicates strong structural control on the drainage pattern while the lower values indicative of subwater sheds that are not affected by structural disturbances. The mean bifurcation ratio (Rbm) may be defined as the average of bifurcation ratios of all orders (Table 1). In the present case, Rbm varies from2.37 to 3.77 (Table 2) and all sub-watersheds fall under normal basin category (Strahler, 1957).

Relief Aspect

The relief measurements like relief ratio, basin length and total relief are tabulated in Table 2.

Relief Ratio

The elevation difference between the highest and lowest points on the valley floor of a sub watershed is

known as the total relief of that sub watershed. The relief ratio (Rh) of maximum relief to horizontal distance along the longest dimension of the basin parallel to the principal drainage line is termed as relief ratio (Schumm, 1956) (Table 1). According to him, there is direct relationship between the relief and channel gradient. There is also a correlation between hydrological characteristics and the relief ratio of a drainage basin. The Rh normally increases with decreasing drainage area and size of sub-watersheds of a given drainage basin (Gottschalk, 1964). The values of Rh are given in Table 2 and ranges from 0.004 to 0.017. It is noticed that the high values of Rh indicate steep slope and high relief (1000 m), while the lower values indicate the presence of basement rocks that are exposed in the form of small ridges and mounds with lower degree of slope (Table 2).

Aerial Aspect

Different morphometric parameters like drainage density, texture ratio, stream frequency, form factor, circularity ratio, elongation ratio and length of overland flow have been discussed in detail and are presented in Table 2.

Drainage density (Dd)

The drainage density is an expression of the closeness or spacing of channels (Horton, 1932). The significance of drainage density is recognized as a factor determining the time travel by water (Schumm, 1956). The measurement of Dd is a useful numerical measure of landscape dissection and runoff potential (Chorley, 1969). On the one hand, the Dd is a result of interacting factors controlling the surface runoff on the other hand, it is itself influencing the output of water and sediment from the drainage basin (Ozdemir and Bird, 2009). Dd is known to vary with climate and vegetation, soil, rock properties, relief and landscape evolution processes (Kelson and Wells, 1989; Oguchi, 1997; Moglen et al., 1998; Srinivasa et al., 2004). The amount and type of precipitation influences directly the quantity and character of surface run-off. An area with high precipitation such as thundershowers loses greater percentage of rainfall as run-off resulting in more surface drainage lines. Amount of vegetation and rainfall absorption and infiltration capacity of soils, which influences the rate of surface run-off, affects the drainage texture of an area. The similar condition of lithology and geologic structures; semi-arid regions have finer drainage density texture than humid regions. According to Nag (1998), low drainage density generally results in the areas of highly resistant or permeable subsoil material, dense vegetation and low relief. High drainage density is the resultant of weak or impermeable subsurface material, sparse vegetation and mountainous relief. Low drainage density leads to coarse drainage texture while high drainage density leads to fine drainage texture.

In the present area, the drainage density varies between 0.21 and 0.4 km/km^2 indicating low drainage density (Table2). It is suggested that this low drainage density indicates the region has highly permeable subsoil. Moreover the study area has low rainfall due to semiarid conditions.

Stream Frequency

The stream frequency (Fs) or channel frequency or drainage frequency of a basin may be defined as the total number of stream segments within the basin per unit area_(Horton, 1945). Hypothetically, it is possible to have the basin of same drainage density differing in stream frequency and basins of same stream frequency differing in drainage density. Table 2 shows Fs for all sub-watersheds of the study area. In the area it is noted that the Fs exhibits positive correlation with the drainage density values of the sub-watersheds indicating the increase in stream population with respect to increase in drainage density.

Drainage Texture

The drainage texture ratio is considered as one of the important concept of geomorphology which shows the relative spacing of the drainage lines (Chorley et al., 1957). According to Horton (1945), Rt is the total number of stream segments of all orders per perimeter of that area (Table 1). He recognized infiltration capacity is the single important factor which influences Rt and considered drainage texture which includes drainage density and stream frequency. Smith (1950) has classified drainage density into five different textures. The drainage density less than 2 indicates very coarse, between 2 and 4 is related to coarse, between 4 and 6 is moderate, between 6 and 8 is fine and greater than8 is very fine drainage texture. In the present area, the drainage density (Table 2) is of very coarse to coarse drainage texture.

Form Factor

Quantitative expression of drainage basin outline form through a form factor ratio (Rf), which is the

dimensionless ratio of basin area to the square of basin length (Horton, 1932). (Table 1). From Table 2 it is observed that the Rf varies between 0.2 'D' and 1.24 'C' and thus indicates that the 'C' sub-watershed is circular in shape with higher value(1.24) whereas the remaining sub-watersheds are elongated with lower values of form factor.

Circularity Ratio

The circularity ratio (Rc) has been used as a quantitative measure for visualizing the shape of the basin and is expressed as the ratio of basin area 'A' to the area of a circle (Ac) having the same perimeter as the basin (Miller1953; Strahler 1964). It is affected by the lithological character of the basin. The ratio is more influenced by length, frequency (Fs), and gradient of streams of various orders rather than slope conditions and drainage pattern of the basin. It is a significant ratio, which indicates the dendritic stage of a basin. Its low, medium and high values are indicative of the youth, mature and old stages of the life cycle of the tributary basins. In the present study, the Rc (Table 2) ranges from0.37 to 0.62. High Rc 0.53 in 'B' and 'C'subwater sheds and 0.40 in 'A' sub-watershed indicates that they are more or less circular and are characterized by high to moderate relief and drainage system is structurally controlled. The remaining 'D' sub watershed has less than 0.40 indicating that they are elongated.

Elongation Ratio

Elongation ratio (Re) is defined as the ratio of diameter of a circle of the same area as the basin to the maximum basin length (Schumm, 1956). It is a very significant index in the analysis of basin shape which helps to give an idea about the hydrological character of a drainage basin. A circular basin is more efficient in the discharge of run-off than an elongated basin (Singh and Singh, 1997). The values of Re generally vary from 0.6 to 1.0 over a wide variety of climatic and geologic types. Values close to 1.0 are typical of regions of very low relief, whereas values in the range 0.6 - 0.8 are usually associated with high relief and steep ground slope (Strahler, 1964).

The lowest Re (0.51) in case of 'D'sub-watershed indicates, high relief and steep slope, while very high values in 'C' sub-watershed (1.25) indicates that plain land with low relief and low slope. Further, it reveals that the 'C' sub-watershed is circular whereas the remaining sub-watersheds are elongated.

Length of Overland flow

It is the length of water over the ground before it gets concentrated into definite stream channels (Horton, 1945) (Table 1). This factor basically relates inversely to the average slope of the channel and is quite synonymous with the length of sheet flow to a large degree. The length of overland flow (Lg) approximately equals to half of the reciprocal of drainage density (Horton, 1945). Table 2 reveals that the Lg is less in 'C'sub-watershed as drainage density is high in this sub-watershed when compared to remaining sub-watersheds. The computed value of Lg for all sub-watersheds varies from 1.25to 2.38.

CONCLUSION

The drainage basin is being frequently selected as a unit of morphometric analysis because of its topographic and hydrological unity. GIS software have resulted to be of immense utility in the analysis of the Linear and Areal morphometric aspects of the drainage basins. The study reveals that GIS based approach in evaluation of drainage morphometric parameters at river basin level is more appropriate than the conventional methods. GIS based approach facilitates analysis of different morphometric parameters and to explore the relationship between the drainage morphometric and properties of landforms, soils and eroded lands.

The morphometric analysis of the drainage networks of all 4 sub-watersheds exhibits the dendritic to sub dendritic drainage pattern and the variation in stream length ratio might be due to changes in slope and topography.

The study area exhibited the mature stage of streams in 'A' and 'B' sub-watersheds and late youth stage of geomorphic development in remaining sub-watersheds. The variation in values of bifurcation ratio among the sub-watersheds is ascribed to the difference in topography and geometric development. The stream frequencies for all sub-watersheds of the study area exhibit positive correlation with the drainage density indicating the increase in stream population with respect to increase in drainage density. Drainage density is very coarse to coarse texture. Elongation ratio shows that 'C' sub-watershed possesses circular shape, while the remaining marks elongated pattern.

REFERENCES

Abrahams AD (1984) Channel networks: a geomorphological perspective. Water Resour Res 20:161–168

Angillieri MYE (2008) Morphometric analysis of Colangüil river basin and flash flood hazard, San Juan, Argentina. Environ Geol55:107–111

Boulton AG (1968) Morphometric analysis of river basin characteristics. Water Resource Board, U.K, p 227

Costa JE (1987). Hydraulics and basin morphometry of the largest flash floods in the conterminous United States. J Hydrol93:313–338

Clarke JI (1966) Morphometry from maps, Essays in geomorphology, Elsevier Publ. Co., New York. pp235–274

Chorley RJ(1969) Introduction to physical hydrology. Methuen and Co Ltd., Suffolk. 211..

John Wilson JS, Chandrasekar N, Magesh NS (2012) Morphometric analysis of major sub-watersheds in Aiyar and KaraiPottanar Basin, Central Tamil Nadu, India using remote sensing and GIS techniques. Bonfring International Journal of Industrial Engineering and Management Science 2(1):8–15

Horton RE (1945) Erosional Development of Streams and their Drainage Basins; Hydrophysical Approach to Quantitative Morphology. Geol. Soc. Am. Bull., 56: 275-370.

Horton RE (1932) Drainage basin characteristics. Am. Geophys. Union, Trans.; 13:348–352.

Huggett R, Cheesman J (2002) Topography and the environment. Prentice Hall, Pearson Education, Upper Saddle River, NJ, p274

Rastogi RA, Sharma TC (1976) Quantitative analysis of drainage basin characteristics. J Soil Water Conserv India 26(1& 4):18–25

Gardiner V (1975) Drainage basin morphometry. British Geomorphological Research Group, Technical Bulletin no. 14, p 48

Gottschalk LC (1964) Reservoir Sedimentation. In: V. T. Chow (ed), Handbook of Applied Hydrology. McGraw Hill Book Company, New York, Section7-1.

Gregory KJ, Walling DE (1973) Drainage basin form and process: a geomorphological approach. Wiley, New York, p 456

Kelson KI, Wells SG (1989) Geologic influences on fluvial hydrology and bed load transport in small mountainous watersheds, northern New Mexico, USA. Earth Surface Processes Landforms, 14:671–690.

Macka Z (2001) Determination of texture of topography from large scale contour maps. Geogr Bull 73(2):53–62

Magesh NS, Jitheshlal KV, Chandrasekar N, Jini KV (2012) GIS based morphometric evaluation of Chimmini and Mupily watersheds, parts of Western Ghats, Thrissur District, Kerala, India. Earth Sci Inform 5(2):111–121

Mesa LM (2006) Morphometric analysis of a subtropical Andean basin(Tucuman, Argentina). Environ Geol 50(8):1235–1242

Maidment DR (2002) ArcHydro GIS for water resources. ESRI Press, Redlands, 220

Miller VC (1953) A quantitative geomorphologic study of drainage basin characteristics in the ClinchMountain area, Virginia and Tennessee, Project NR 389042, Tech Report 3. Columbia University Department of Geology, ONR Geography Branch, New York

Moussa R (2003) On morphometric properties of basin, scale effects and hydrological response. Hydrol Processes 17:33–58

Moglen GE, Eltahir EA, Bras RL (1998) On the sensitivity of drainage density to climate change. Water Res. Res.; 34:855–862.

Nag SK and Chakraborty S (2003) Influence of Rock Types and Structures in the Development of Drainage Network in Hard Rock Area. J. Indian Soc. Remote Sensing, 31(1): 25-35.

Oguchi T (1997) Drainage density and relative relief in humid steep mountains with frequent slope failure. Earth Surf. Processes Landforms, 22:107–120.

Ozdemir H Bird D (2009) Evaluation of morphometric parameters of drainage networks derived from topographic maps and DEM in point floods. Environ Geol 56:1405–1415

Rinaldo A, Rodriguez-Iturbe I, Rigon R (1998) Channel networks. In: Jeanloz R, Albee AL, Burke KC (eds) Annual review of earth and planetary sciences, vol 26. Annual Reviews, Palo Alto, pp 289–327

SrinivasaVittala s, Govindaiah s, HonneGowda H (2004) Morphometric analysis of sub-watersheds in the pavagada area of Tumkur district, South India using remote sensing and GIS techniques. Journal of the Indian Society of Remote Sensing, Vol. 32, No. 4,

Schumm SA (1956) Evolution of Drainage Systems and Slopes in Badlands at Perth Amboy, New Jersey. Geol. Soc. Am. Bull., 67: 597-646.

Singh S and Singh MC (1997) Morphometric analysis of Kanhar river basin. National Geographical J of India43(1):31–43

Strahler AN (1957) Quantitative analysis of watershed geomorphology. Trans. Am. Geophys. Union, 38: 913-920.

Strahler AN (1964) Quantitative geomorphology of drainage basins and channel networks. In: V. T. Chow (ed), Handbook of Applied Hydrology. McGraw Hill Book Company, New York, section 4-1I.

Smith KG (1950) Standards for Grading Textures of Erosional Topography. Am. Jour. Sci., 248: 655-668.

Topaloglu F (2002) Estimation of instantaneous peak flows in Seyhan river basin using regional regression procedures. Turk J Agric For26:47–55

Verstappen H (1983) The applied geomorphology. International Institute for Aerial Survey and Earth Science (I.T.C), Enschede.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

3, 5-Dichloroanthranilic acid (DCA) - an elicitor induces systemic resistance against downy mildew in pearl millet

Lavanya SN and Amruthesh KN*

Applied Plant Pathology Laboratory, Department of Studies in Botany, Manasagangotri, University of Mysore, Mysuru, India -570006
*Corresponding author E-mail: dr.knamruthesh@botany.uni-mysore.ac.in

Manuscript details:

Received: 23.12.2015
Revised: 21.01.2016
Accepted: 16.02.2016
Published : 10.03.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Lavanya SN and Amruthesh KN(2016) 3, 5-Dichloroanthranilic acid (DCA) - an elicitor induces systemic resistance against downy mildew in pearl millet. *International J. of Life Sciences*, 4(1): 97-106.

Acknowledgement
The authors are grateful to University Grands Commission (UGC) for financial assistance under Major Research Project sanctioned to corresponding author. The facilities provided by the Indian Council of Agricultural Research (ICAR), the Government of India, through the All-India Coordinated Pearl Millet Improvement Program (AICPMIP) is also gratefully acknowledged.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

Seed treatments with synthetic elicitor DCA were evaluated for their potential to induce resistance in pearl millet against downy mildew disease caused by *Sclerospora graminicola Schroet*. Disease resistance in plants is associated with the activation of a wide variety of defense responses that serve to prevent pathogen infection. Pearl millet seeds were treated with DCA at different concentrations viz., 10, 20, 50, 100µM for 4h. Among these, 100 µM was found to be optimum, and recorded significant disease protection compare to other concentrations, which revealed maximum germination of 93.7% and seedling vigor 1480 compare to control seedlings. DCA treatment also exhibited anti-sporulation activity under laboratory conditions. Under In-vivo conditions DCA primed seeds exhibited intense decline in downy mildew incidence with significant protection of 64.1% in 100 µM. Vegetative growth parameter studies revealed the enhanced growth of pearl millet when compare to control. Restorative activity of Seedlings sprayed with DCA solution reduced the severity of disease incidence of 33.9% with significant increase in disease protection. Time-gap studies revealed, seed treatment increased disease resistance four days after inoculation. Additionally, Pearl millet seedlings raised after seed treatment with 100µM DCA showed an increased level of the defense-related enzymes: Phenylalanine ammonia-lyase activity and Lipoxygenase, compared with the untreated control. The significant PAL activity was observed at 12h with 4.05U hai and enhanced LOX activity of 5.7U hai was found at 48h.

Keywords: DCA, Pearl Millet, *S. graminicola,* PAL, LOX.

INTRODUCTION

Plants can activate a resistance response upon recognition of a potential pathogen or its products both locally and systemically. The defense response can be accelerated or enhanced by the application of specific compounds, which act as an elicitor or inducer (Lyon *et al*., 1995; Kuc, 2001; Gozzo, 2003). Plant or seed treatment with elicitors often results in a resistance against different pathogens simultaneously (Oostendorp *et al*., 2001). The plant's immunity can be prompted by initial localized infection with pathogens that cause lesions including host cell death (Ross, 1966). The resistance induction in plants results in the

development broad spectrum of immunity in non-infected tissues against a comprehensive range of plant pathogens (viruses, bacteria and fungi) (Vallad and Goodman, 2004; Walters *et al*., 2005; Kuc, 2006). Induced resistance by chemicals is also a promising approach to prevent diseases caused by soil-borne pathogens (Okubara *et al*., 2005). Benzo [1,2,3] thiadiazole derivatives have been shown to mimic the biological activation of systemic acquired resistance by necrogenic pathogens (Kunz *et al.,*1997), synthetic analogue of salicylic acid like Acibenzolar-S-methyl (BTH, ASM), protected cantaloupe against *Colletotrichum lagenarium* and cucumber mosaic virus (CMV) (Smith-Becker *et al*., 2003). Extensive range of chemical agents have been shown to trigger innate defense mechanisms via host-pathogen interactions at physiological, biochemical and molecular levels (Vallad and Goodman, 2004; Kogel and Langen, 2005).

Pearl millet [Pennisetum glaucum (L.) R. Br.] Is one of the most important staple food in the semiarid regions of the world. In India, pearl millet is the third most important cereal crop and is grown over 10 million hectares mainly as a rain fed crop with an annual production of 9.5 million tonnes. The major constraint for pearl millet production is downy mildew disease caused by the obligate, biotrophic, oomyceteus pathogen *Sclerospora graminicola* (Sacc.) Schroet. Disease causes systemic infection in pearl millet that manifests itself as foliar symptoms and malformation of the panicles resulting in severe grain loss. Production resulting upto 80% yield loss (Howarth and yadav, 2002). One of the newer and ecofriendly approaches to manage this disease is by induction of resistance by using biotic and abiotic elicitors. The effect of abiotic and biotic elicitors involves biochemical changes in the host metabolism that may play a role in limiting plant infection.

Abiotic inducers include chemicals which act at various points in the signaling pathways involved in disease resistance. Induced defenses are often triggered by the recognition of conserved pathogen-associated molecular patterns (PAMPs), resulting in PAMP-triggered immunity (PTI) (Gomez-Gomez and Boller, 2002). 3, 5-dichloroanthranilic acid (DCA) a synthetic elicitors, competently induces defense responses to two the phytopathogens (*Hyaloperonospora parasitica* and *Pseudomonas syringe)* by concurrently activating two distinct branches of the plant defense signaling network. (Knoth *et al.,* 2009) DCA and the SA analog INA to be functionally distinct with regard to their dependency on NPR1 and the kinetics of their activities. SA signaling is partially dependent on NPR1 (Non expresser of Pathogenesis- Related genes1), a transcriptional cofactor that is required for the activation of multiple defense genes (Dong, 2004). DCA acts transiently and is only partially dependent on (NPR1) Microarray analyses revealed a cluster of 142 DCA- and INA-responsive genes that show a pattern of differential expression coinciding with the kinetics of DCA-mediated disease resistance (Knoth *et al.,* 2009). *Arabidopsis thaliana* Immune responses against *Hyaloperonospora parasitica* are somewhat facilitated by transcriptional upregulation in response to (LURP) cluster of plant defense genes. Resistance in plants is influenced by Elicitation of Induced defense to recognition of pathogen effectors by Leu-rich repeat-containing plant resistance (R) proteins by making the pathogen avirulent and the plant resistant (Jones and Dangl, 2006). The current study was aimed to investigate the synthetic elicitor 3, 5-dichloroanthranilic acid (DCA) could able to trigger disease resistance and defense enzymes in pearl millet for imparting systemic resistance against downy mildew pathogen. Multiple mechanisms are responsible for induced or acquired resistance, and different types of interactions between pathways were studied.

MATERIALS AND METHODS

Host

Seeds of pearl millet cultivars 7042S highly susceptible to the downy mildew pathogen were obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patencheru, Hyderabad, Andhra Pradesh, India, were used throughout the study.

Pathogen source and inoculum preparation

Downy mildew pathogen *S. graminicola* was isolated from susceptible cv.7042S. The susceptible pathogen was maintained on the same cultivar prior to use under greenhouse conditions (temperature of 22 ± 2 ºC and relative humidity of 80 %). Pearl millet leaves showing prolific sporulation of *S. graminicola* on the abaxial side were collected in the evening hours. The collected leaves were thoroughly washed under running tap water to remove dust and old sporangia. Then the collected leaves were blot dried and cut into

smaller pieces, and placed in moist chamber for sporulation. The next morning, Fresh sporangia were collected and zoospores were released into sterile distilled water. Zoospore concentration was adjusted to 4×10^4/ml using hemocytometer and used as inoculum for all inoculation experiments (Safeeulla, 1976).

In-vitro studies

Preparation of inducer and seed treatment

DCA obtained from Sigma DCA solutions were prepared by dissolving 10µM, 20 µM, 50 µM, 100 µM in DMSO (1µl/ml) of 100mL of sterile distilled water and kept for constant agitation for 2h for complete dissolution. For seed treatment, pearl millet seeds were surface sterilized with 0.02% mercuric chloride solution for 4-5 min and then thoroughly rinsed in sterile distilled water. Seeds were submerged in 10ml of inducer solution with different concentrations viz., 10µM, 20 µM, 50 µM, 100 µM. Treated seeds were incubated at 25^0C in a rotary shaker for 4h to facilitate the seed treatment. For the same time interval, Seeds treated with sterile distilled water served as control.

Influence of DCA on seed germination and seedling vigor of pearl millet

Seed germination and seedling vigor were evaluated by treating Seeds with different DCA concentrations of 10µM, 20 µM, 50 µM, 100 µM for 4 h. Germination tests were carried out by the paper towel method (ISTA, 2003). Seeds treated with sterile distilled water for the same duration served as controls. Treated seeds and controls were planted onto paper towels. Treated seeds of pearl millet were placed equidistantly on the paper (100 seeds/ paper towel). The towels were then rolled and wrapped with polythene to prevent drying and incubated for 7 days. The number of seeds germinated were counted and represented in percentage of germination. At the end of 7 days, Seedling vigor was analyzed (Abdul Baki and Anderson, 1973) by measuring the length of the shoot and root of individual seedlings. The experiment was carried out three times with four replicates of 100 seeds each. The vigor index was calculated using the formula:

$$\text{Vigor Index} = (\text{Mean root length} + \text{Mean shoot length}) \times (\%\ \text{Germination})$$

Anti-oomycete activity of DCA

Downy mildew infected disease leaves were collected from infected plants grown in greenhouse. Leaves were washed in sterile distilled water, excess of water was removed and blot dried. Leaves were cut in to small discs of 10 mm diameter size by using sterilized cork borers and consequently Immersed in different concentration of DCA solution for 5-10 mins (Wafaa and Haggag, 2002; Musetti *et al*., 2006 and Deepak, 2005). One set of leaf disc dipped in distilled water for the same interval served as control. The treated leaf discs were incubated overnight in a moist chamber for sporulation. After incubation, leaf disc were analyzed under stereo binocular microscope for sporulation and sorted as full inhibition (++), moderate inhibition (+) and no inhibition (-) compare to control set.

In-vivo studies

Effect of seed treatment on pearl millet downy mildew disease

The treated seeds were sown in earthen pots filled with autoclaved soil, sand and manure (in the ratio of 2:1:1). The experiment consisted of four replicates, 10 pots in each replication with 10 seedlings per pot (12-14in diameter) and experiment was repeated thrice. Treated pots were arranged in a randomized complete block design. Seeds treated with sterile distilled water served as control (non-treated).

Seeds treated with the systemic fungicide metalaxyl formulation (Apron 35 SD at 6 g/kg concentration) served as a chemical control. Zoospore suspension of *S. graminicola* (4×10^4zoospores/ml) was prepared as described before. Emerging seedlings at coleoptile stage were challenge-inoculated by the whorl inoculation (Singh and Gopinath, 1985). In the whorl inoculation method, droplets of *S. graminicola* zoospores were dropped onto the leaf whorl of the emerging seedlings and allowed to flow down to the base. Inoculated plants were maintained under greenhouse conditions (20–26 ^{0}C temperature with 90–95% RH) and observed regularly for the development of disease symptoms. The plants were assessed disease when they showed any one of the typical symptoms of downy mildew like sporulation on the abaxial leaf surface, stunted growth, chlorosis, or malformation of the ear heads. Percentage Downy mildew disease incidence was recorded at 30 DAS and final counts were made at 60 DAS as percentage of plants showing symptoms of downy mildew disease.

$$\text{Disease Protection} = \frac{\text{Downy mildew disease incidence in control- Downy mildew disease incidence in treated plants}}{\text{Downy mildew disease incidence}} \text{X100}$$

Downy mildew disease protection was calculated using above formula,

Influence of DCA on growth parameters of pearl millet under greenhouse conditions

Evaluation of growth promotion under greenhouse conditions was carried out in pearl millet cv. 7042S seeds treated DCA for 6 h time duration sown in earthen pots filled with autoclaved soil, sand and manure (in the ratio of 2:1:1) The experiment consisted of four replication with 100 seedlings each and was repeated three times. Plants were maintained under greenhouse conditions (20–26 ^{0}C temperature with 90–95% RH) and observed regularly for the development of disease symptoms. Seeds treated with SDW served as control. After 30 days of sowing, seedling height, shoot fresh and dry weight, leaf surface area and number of basal tiller per plant were measured and recorded accordingly.

Restorative activity of DCA against downy mildew

Susceptible cultivar 7042S seeds were sown to earthen pots and maintained under greenhouse condition as explained before The different concentration of DCA solution were applied to15 day old sporulating downy mildew infected plants. DCA solutions were smeared on to abaxial leaf surface with soft brush constantly for 3 days. One set of infected plants applied with distilled water served as control. The experiment was carried out three times with four replicates of 100 plants each. After 5 days, Inhibition of sporulation were recorded and tabulated.

Effect of DCA on induced resistance by Time gap studies in pearl millet

Spatio-temporal time gap studies were carried out between inducer treatments and the pathogen inoculation (Amruthesh *et al.,* 2005) in order to understand the systemic nature of disease protection offered by seed treatment. The pearl millet seeds (7042S) treated with DCA for 4h were sown in earthen pots filled with autoclaved soil and maintained as explained earlier along with control seeds (seeds treated with sterile distilled water). Pots were arranged in a randomized complete block design. After Two days, seedlings at coleoptile stage were challenge inoculated with zoospore suspension of *S. graminicola* (4 x 10^4 zoospore/ml) following the whorl inoculation method and with a time gap of 1, 2, 3, 4, 5 and 6 days in different set of plants. Plants were maintained under greenhouse conditions and observed regularly for the expression of downy mildew disease. The experiment consisted of four replicates with 100 seedlings each and was repeated three times. At the end of 60 days, Disease incidence and protection of downy mildew disease was calculated as mentioned above.

Biochemical studies

Sampling of seedlings and enzyme extraction

Two-day-old seedlings of susceptible (7042S) and 100µl of DCA treated seeds along with control seedlings (sterile distilled water treated) were root dip inoculated with the zoospore suspension of *S. graminicola* (4 X 10^4 zoospores/ml). The seedlings were harvested at different time intervals 0, 3, 6,9,12, 24, 48 and 72 h post inoculation (hpi) were immediately stored at -80º C prior to analysis and used for biochemical studies. One gram fresh weight of Seedlings were weighted from harvested seedlings and were ground to a fine powder in liquid nitrogen and by homogenizing in different buffers (1ml/g seedlings) for extraction of different enzymes at 4^{0}C. These were centrifuged at 12,000 g for 10 min at 4^{0}C and the supernatants were used for further enzyme assays. Protein content in the crude extracts was estimated by Dye binding method (Bradford, 1976) using BSA (Bovine serum albumin) (Sigma) as a standard.

Phenylalanine ammonia-lyase (PAL, E.C. 4.1.3.5)

PAL enzyme was extracted with 25mM Tris HCL buffer (P^H 8.8). PAL activity was assayed according to the procedure of Beaudoin-Eagan and Thorpe (1985). One hundred µl of extracts were mixed with reaction mixture of 900µl of 50mM L-phenylalanine and 100mM Tris HCl buffer solution. The mixture was placed in a water bath for 120min at 40^{0}C. The reaction was stopped by adding 60µl of 5N HCL. PAL enzyme activity was measured spectrophotometrically at a wavelength of 290nm and expressed as the amount of t-cinnamic acid formed from L-phenylalanine per mg of protein per min.

Lipoxygenase (LOX) (Linoleate: oxygen oxidoreductase, EC 1.13.11.12)
Enzyme activity was measured by following the procedure of Borthakur *et al*. (1987). The substrate for assay was prepared according to the method described by Axelrod *et al*. (1981). 28 mg of Linoleic acid was mixed with an equal volume of Tween-20 in 2 mL of distilled water.

50 µL of 2N NaOH was added to obtain a clear solution and volume of the solution was made up to 10 mL with distilled water. Each time the substrate was prepared fresh and used for the enzyme assay. The reaction mixture contained 0.3 mL of substrate with 3 mL of 0.2 M Sodium phosphate buffer (pH 6.5). The reaction was initiated by adding 10 µl the enzyme extract. The activity was determined spectrophotometrically by monitoring the appearance of the conjugated diene hydroperoxide at 234 nm. The enzyme activity was expressed as a change in the absorbance (δ 234) /mg protein/min. The change in absorbance at 234 nm was recorded for three minute.

Statistical analysis
Data from four replicates were analyzed for each experiment and subjected to arcsine transformation and analysis of variance (ANOVA) using SPSS Inc. 16.0. Significant effects of treatments were determined by F values (P = 0.05). Treatment means were separated by Tukey's honestly significant differences (HSD) test.

RESULTS

Influence of DCA on seed germination and seedling vigor
Compared to untreated control, Seeds treated with DCA with different concentration showed significant enhancement of seed germination and vigor index to varying degrees. Highest percentage of seed germination of 93% and 90% was recorded in 100µM and50µM respectively (Fig.1). Similar tendency was also noticed for vigor index in all DCA treated seeds. Maximum seedling vigor of 1480 was recorded in 100µM followed by 1398 in 50µM treated seeds. The untreated control recorded 85% germination and 983 seedling vigor.

Anti-oomycete activity of DCA
Pearl Millet infected leaf disc when treated with different concentrations of DCA exhibited anti-sporulant activity compare to distilled water treated leaf disc (control). Leaf disc when treated with 100µM showed complete inhibition of sporulation followed by 50µM of DCA which showed partial inhibition when compare to control (data not shown). There was a gradual decline in sporulation on the treated leaf discs.

Effect of different concentrations of DCA on potential to activate resistance against downy mildew under greenhouse conditions

Effect of seed treatment on pearl millet downy mildew disease under greenhouse conditions
Seed treatment with DCA at different concentration protected pearl millet plants against downy mildew disease when compared to the untreated control. Overall pictorial assessment of treatments indicated obvious difference in the disease incidence when compared with the control. Highest downy mildew incidence was observed in control with 89.5% followed by 55.2% incidence in 10µM treatment. Among treated plants, least disease incidence was recorded in Metalaxyl with 8.6% and 32.1% in 100µM treated seeds compare to control (Fig. 3). However highest disease protection was recorded in Metalaxyl treated seeds with 90.3% protection. Among treated seeds, disease protection of 64.1, 59.4% was recorded in 100µM and 50µM compare to control sets respectively.

Table.1. Effect of seed treatment on growth parameters of pearl millet plants under greenhouse conditions.

Concentration (µM)	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Leaf surface area (cm^2)	No. of tillers/plant
10	29.1±0.2^d	12.8±0.1^b	2.9±0.1^c	31.9±0.3^d	2.6±0.3^b
20	33.4±0.2^c	13.2±0.3^b	3.4±0.05^b	36.2±0.2^c	3.3±0.3^{ab}
50	37.2±0.4^b	14.7±0.2^a	3.7±0.05^b	38.9±0.2^b	3.6±0.3^{ab}
100	39.5±0.2^a	15.6±0.1^a	4.1±0.05^a	40.6±0.05^a	4.2±0.3^a
Control	23.6±0.4^e	8.5±0.1^c	2.2±0.05^d	26.3±0.3^e	3.0±0^b

Values are means of four independent replicates. Means followed by the same letter(s) within the column are not significantly different according to Tukey's HSD test (P = 0.05).

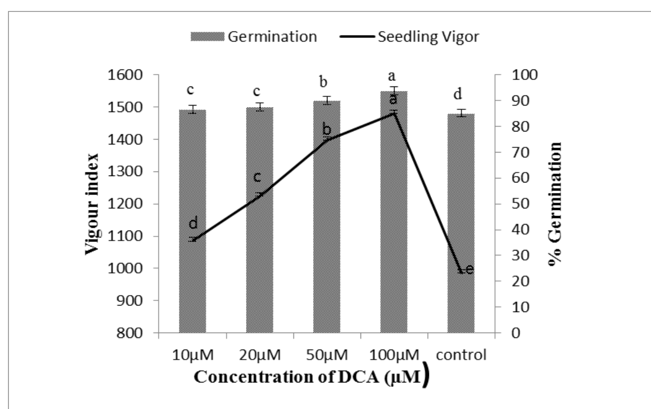


Fig.1. Influence of DCA seed treatment on Germination and Seedling vigor. Values are means of four independent replicates. Bars represent standard errors. Means followed by different superscripts in the columns are significantly different according to Tukey's HSD test (P = 0.05).

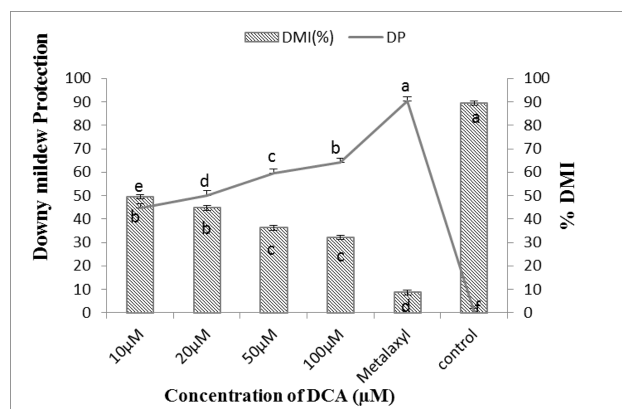


Fig.2. Efficacy of seed treatment with different concentration of DCA on Percentage of downy mildew incidence (DMI) and downy mildew disease protection (DMP) under greenhouse conditions. Values are means of four independent replicates. Bars represent standard errors. Means followed by different superscripts in the columns are significantly different according to Tukey's HSD test (P = 0.05).

Influence DCA on growth parameters of pearl millet under greenhouse conditions

Seed treatment with DCA not only resulted in increased disease resistance against *S. graminicola*, but also considerably enhanced the vegetative growth parameters upon inducer treatment under greenhouse conditions. Among all the concentrations, the maximum increase was recorded in 100µM treatment with all growth parameter like plant height was increased by mean up to 39.5 cm, shoot fresh weight and dry weight were also increased with 15.6 and 4.1gm, leaf surface area of 40.6cm^2 and the number of tillers in control was three whereas 100µM DCA treated plants increased the mean to 4.6 (Table.1). On overall assessment of growth parameter remained found to be reliant on concentration with increase in concentration of the treatment.

Restorative activity of DCA against pearl millet downy mildew

Inducer (with different concentrations) when applied to abaxial surface of leaves showed significant decline in disease reaction when compare to distilled water smeared plants (Fig.4). 100µM DCA applied plants exhibited disease incidence of 33.9% followed by 50µM with 45.4%. Metalaxyl smeared plants showed disease frequency of 6.8% and highest disease incidence of 92.7% was noticed in distilled water treated plants respectively.

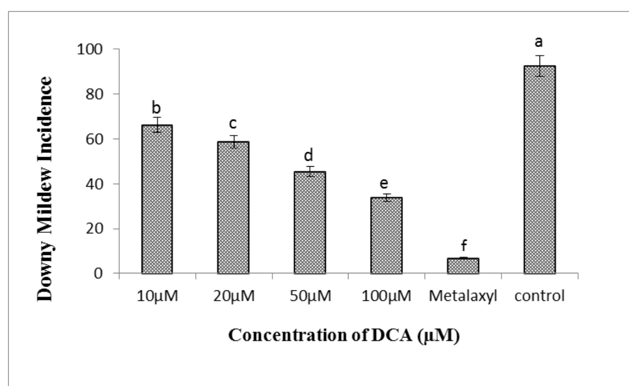


Fig.3. Translaminar activity of DCA in infected pearl millet leaves as indicated by Downy mildew disease Incidence (DMI). Values are means of four independent replications. Bars represent standard errors. Means with different superscripts are significantly different according to Tukey's HSD test (P = 0.05).

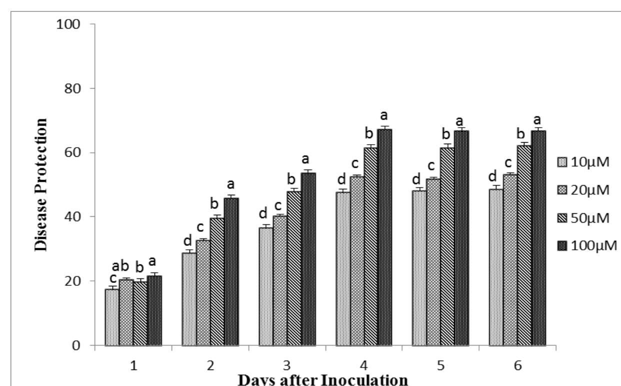


Fig.4. Influence of Seed treatments on Spatio-temporal effect during induction of resistance. Values are means of four replicates. Bars represent standard errors. Means followed by the same letter(s) within the column are not significantly different according to Tukey's HSD test (P = 0.05).

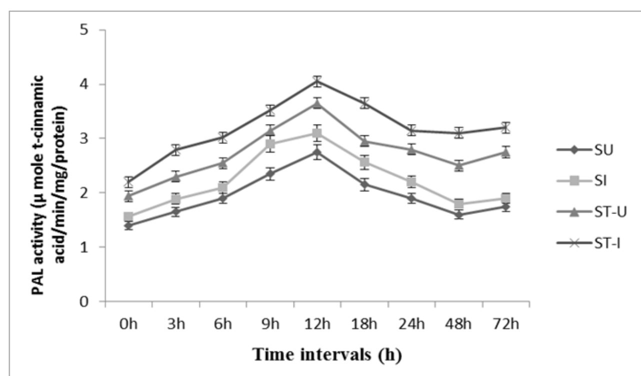


Fig.5. Induction of Phenylalanine ammonia-lyase activity in pearl millet seedlings treated with different concentrations of DCA on inoculation with *Sclerospora graminicola*. STU-susceptible treated uninoculated, STI – susceptible treated inoculated, SU - susceptible uninoculated, SI – susceptible inoculated. Bars indicate standard errors [Tukey's HSD test (P = 0.05)].

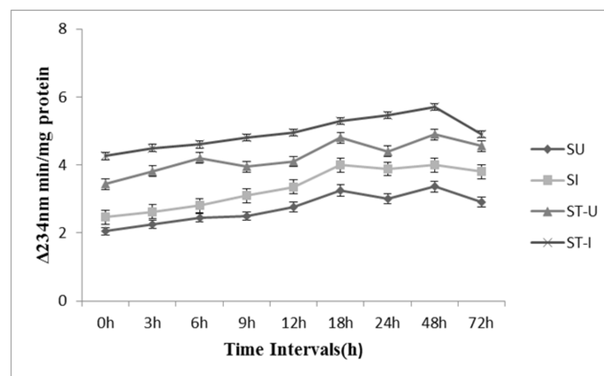


Fig.6. Estimation of Lipoxygenase activity at different time intervals after inoculation. STU – susceptible treated uninoculated, STI – susceptible treated inoculated, SU - susceptible uninoculated, SI – susceptible inoculated. Bars indicates standard errors [Tukey's HSD test (P = 0.05)].

Effect of DCA on induced resistance by Time gap studies in pearl millet

Spatio-temporal studies were assessed for demonstrating the nature of resistance induction by using different concentrations of DCA. Seed treatment with 100µM showed the maximum increase in disease resistance from 21 to 67% on four days after inoculation (Fig.5). Seedlings inoculated on the fourth day established maximum disease protection against downy mildew pathogen. The disease protection was found to be stable at fifth and sixth day and induction of resistance was maintained at the same levels. Seed treatment and seedling inoculation with the downy mildew pathogen were studied for system nature of disease resistance at different time intervals (time gas of 1-6days) respectively.

Influence of DCA seed treatment on enzyme activity

Time course study of PAL activity

Estimation of PAL activity in DCA treated and untreated control seedlings at different time intervals recorded consecutive increase in activity. PAL activity was determined by measuring the conversion of L-phenylalanine to t-cinnamic acid in enzyme assays. The PAL activity increased gradually from 2 hpi and reached up to 72 hpi. In treated seedlings, the maximum activity was observed at 12 hpi and there was a substantial decrease in activity at 24 hpi. The maximum activity of 4.05 U was observed at 12 hpi in induced treated which was 1.9-fold higher than untreated control followed by 3.65 U at 18 hpi. The results revealed that, there is an increase in enzyme activity in DCA treated seedlings upon inoculation showed significantly increase when compared to control inoculated seedlings.

Time course study of LOX activity

LOX activity was more in DCA treated pearl millet seedlings than in untreated control. Seedlings upon challenge inoculation showed a steady increase in enzyme activity from early hours and reached a maximum at 48 hpi compared to the un-inoculated control. In induced treated, 5.7 U of maximum activity were recorded at 48 hpi followed by 5.4 U at 24 hpi. The enzyme activity increased by 2.1-fold at 48 h after inoculation compare to control. In uninoculated seedlings the enzyme activity declined at all the intervals when compared to inoculated seedlings.

DISCUSSION

The sever susceptibility of pearl millet to downy mildew oveing tough challenge to the scientific community aiming towards designing rapid and cheaper management strategy to control disease and yield loss. Number of synthetic and natural chemicals had been reported to enhance host resistance against pathogen infection systemically (Hong *et al*., 1999). Our findings suggest the possible use of DCA a synthetic abiotic inducer to protect pearl millet against the downy mildew disease. In pearl millet downy mildew system certain abiotic elicitors have already been reported like Benzothiadiazole, $CaCl_2$ and H_2O_2

(Geetha and Shetty, 2002), β-aminobutyric acid (Shailashree *et al.,* 2001), Chitosan (Manjunath *et al.,* 2008), Proline (Raj *et al.,* 2004), Cerebroside (Deepak *et al.,* 2003), Elexa (Sharathchandra *et al.,* 2004) and L-methionine (Sarosh *et al.,* 2005).

Seed treatments with DCA enhanced seed germination and seedling vigor of pearl millet under laboratory conditions. None of the DCA concentration caused any phytotoxicity in pearl millet. The gradual decrease in sporulation was observed with application of the different concentrations of DCA solution as compared to control by leaf disc assay (Deepak *et al.,* 2005). Sporulation decreases as the DCA concentration increases. During greenhouse experiments it was observed that, the seed treatment with different concentrations of DCA reduced the disease incidence and offered highest level of disease protection. The systemic and durable nature of resistance was demonstrated by the spatio- temporal separation of the inducer against pathogen inoculation. Studies concluded that a minimum of 4-day time gap prior to pathogen inoculation was necessary for treatment to elicit maximum protection against downy mildew disease. The seeds treated with DCA also enhanced vegetative growth of pearl millet. Seedlings sprayed with DCA also exhibited high level of restorative activity with decline in disease incidence which revealed significant disease protection. The effectiveness of Cyazofamid was reported by earlier workers for their preventive and sporulation inhibitory activity and also for their curative activity against downy mildew in pearl millet (Sudisha *et al.,* 2007).

Induction of resistance has been accessed through signaling pathways using biochemical indicators in the form of induction of defense related enzymes that are activated upon pathogen infection. With the evidence of biochemical studies, we account the involvement of PAL and LOX during the pearl millet and DM disease interaction. Phenylalanine is a critical precursor of a cascade of defense reactions leading to ISR (Dempsey *et al.,* 1999) and is also reported as molecular signals during recognition of pathogen by the host (Lynn and Chang, 1990) the main role of PAL activity in phenylpropanoid metabolism is by converting L-phenylalanine to trans-cinnamic acid (Hahlbrock and sched, 1989). Increased expression of PAL was observed in all time intervals and significant increase was observed after the inoculation. Comparatively highest PAL as recorded in induced resistant seedling at 12 hai. The maximum activity of 4.05 U was observed in treated inoculated seedlings, which was 2-fold higher than control. Lipoxygenase are dioxygenases that catalyze the hydroperoxidation of polyunsaturated fatty acids or their esters that contain a cis, cis-1, 4-pentadiene moiety. In higher plants, the natural substrates for these enzymes are linolenic and linoleic acids (Siedow, 1991; Conconi *et al.,* 1996). Many studies have shown increased LOX activity in plant tissues and cells in response to plant pathogens (Ohta *et al*., 1991). In corresponds to our study, the Lox activity increased with increase in time with a peak at 48 hours after inoculation. However, the increase in Lox activity was significantly higher in induced resistant seedlings with 5.7 U, which was 2.1 fold higher from that of the untreated control seedlings. Lipoxygenase activity has been reported in tomato leaves (*Lycopersicon esculentum* Mill.) for the induction of resistance in response to plant pathogen *Pseudomonas* (Koch *et al*., 1992). Chemically induced LOX (WCI- 2) gene expression correlated with the onset of resistance against *Erysiphe graminis* f.sp. *tritici* in wheat (Gorlach *et al*., 1996). Induction of LOX activity and resistance of the plant has been shown in tobacco infected with *Erysiphe cichoracearum* (Lupu *et al*., 1980) and also induction of resistance against *Magnaporthe grisea* in rice (Ohta *et al*., 1991).

DCA, which acts as plant defense activator that triggers a defined aspect of the plant defense network. Screens for proteins directly targeted by DCA or operating downstream from DCA perception to reveal new components of the plant immune response was reported by earlier workers (Knoth *et al.,* 2009). The defining features of DCA-type elicitors are the presence of the 3- and 5-position chlorines and an amino group at position 2. There are no much reports on application of DCA for defense induction against. DCA induced resistance to two phytopathogens *H. parasitica and Pseudomonas syringae* in *Arabidopsis thaliana.* In microarray analyses, the induction of resistance by DCA and role of NPR1 in were estimated after 48 h of DCA treatment with npr1 mutant. Of the 137 DCA-inducible ACID genes, 20% exhibited NPR1-independent transcriptional up-regulation (Knoth *et al.,* 2009). DCA and INA turned out to be more complex, because their efficiencies in inducing defense activation. Both INA and DCA can formally be considered as two representatives of a variety of related defense-inducing molecules. The defense

activation is completely obstructed in npr1 which discriminates DCA from SA, INA, and BTH (Lawton *et al.,* 1996; Lipinski *et al.,* 1997; Knoth *et al.,* 2007). GO and microarray analyses reveals the genes which are upregulated by DCA treatment and also genes associated with defense response. ACID cluster as a set of genes strictly associated with defense activation. Cluster contains many known defense-related genes and is highly enriched for genes associated with calmodulin binding and kinase activity. Among the upregulated ACID genes are six genes encoding WRKY transcription factors, which have been associated with plant immune responses (Eulgem and Somssich, 2007).

CONCLUSION

Chemicals that transiently activate plant immunity may be beneficial in combating virulent pathogens that threaten crops only during a limited period of time. A transiently active compound like DCA may allow fine-tuned control of defense induction coordinated with the plant's needs, thereby decreasing unwanted side effects caused by long-term defense activation. The present study revealed the efficacy of synthetic elicitor DCA was tested against *S. graminicola* in pearl millet and the evaluation of potentials to imply this compound into the disease management programs.

REFERENCES

Abdul Baki AA and Anderson JD (1973) Vigour determination in soybean seed by multiple criteria. *Crop Science*, 13(6): 630–633.

Amruthesh KN, Geetha NP, Jorgensen HL, De Neergaard E and Shetty HS (2005) Unsaturated fatty acids from zoospores of *Sclerospora graminicola* induce resistance in pearl millet. *European Journal of Plant Pathology*, 111(2): 125-137.

Axelrod B, Cheesbrough TM and Laakso S (1981) Lipoxygenase from soybeans. *Methods Enzymology*, 71: 441-451.

Beaudoin-Eagan LD and Thorpe TA (1985) Tyrosine and phenylalanine ammonia-lyase activities during shoot initiation in tobacco callus cultures. *Plant Physiology*, 78(3): 438–441.

Borthakur AB, Bhat BG and Ramasoss CS (1987) The positional specifications of the oxygenation of linoleic acid catalysed by two forms of lipoxygenase isolated from Bengal gram (Cicer arietinum). *Journal of Bioscience*, 11:257–263.

Bradford MM (1976) A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry,* 72(1): 248–254.

Conconi A, Miquel M and Ryan CA (1996) Intracellular levels of free linolenic and linoleic acids increase in tomato leaves in response to wounding. *Plant Physiology*, 111(3):797– 803.

Deepak SA, Nirajan Raj S, Umemura K, Kono T and Shetty HS (2003) Cerebroside as an elicitor for inducing resistance against downy mildew disease of pearl millet. *Annals of Applied Biology*, 143(2): 169–173.

Deepak SA, Oros G, Sathyanarayana SG, Shetty NP, Shetty HS and Shashikanth S (2005) Antisporulant activity of leaf extracts of Indian Plants against *Sclerospora graminicola* causing downy mildew disease of pearl millet. *Archives of Phytopathology and Plant Protection*, 38(1):31-39.

Dempsey DM, Shah J and Klessig DF (1999) Salicylic acid and disease resistance in plants. *Critical Reviews in Plant Sciences*, 18(4): 547-575.

Dong X (2004) NPR1, all things considered. *Current Opinion in Plant Biology*, 7:547–552.

Eulgem T and Somssich IE (2007) Networks of WRKY transcription factors in defense signaling. *Current Opinion in Plant Biology* , 10(4): 366–371.

Geetha HM and Shetty HS (2002) Induction of resistance in pearl millet against downy mildew disease caused by *Sclerospora graminicola* using benzothiadiazole, calcium chloride and hydrogen peroxide. A comparative evaluation. *Crop Protection*, 21(8): 601-610.

Gomez-Gomez L and Boller T (2002) Flagellin perception: a paradigm for innate immunity. *Trends in Plant Science*, 7(6): 251–256.

Gorlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, Kogel KH, Oostendorp M, Staub T, Ward E and Kessman H (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in barley. *The Plant Cell*, 8(4): 629–643.

Gozzo F (2003) Systemic acquired resistance in crop protection: from nature to a chemical approach. *Journal of Agricultural and Food Chemistry*, 51(16): 4487-4503.

Hahlbrock K and Scheel D (1989) Physiology and molecular biology of phenylpropanoid metabolism. *Annual Review of Plant Biology*, 40(1):347–369.

Hong JK, Hwang BK and Kim CH (1999) Induction of local and systemic resistance to colletotrichum coccodes in pepper plants by dl-β-Amino-n-Butyric Acid. *Journal of Phytopathology*, 147(4):193–198.

Howarth CJ and Yadav RS (2002) Successful marker assisted selection for drought tolerance and disease resistance in pearl millet. *IGER Innovations*, 6:18-21.

ISTA (2003) International Rules for Seed Testing. International Seed Testing Association (Chapter V).

Jones JD and Dangl JL (2006) The plant immune system. *Nature*, 444(7117): 323–329.

Knoth C, Ringler J, Dangl JL and Eulgem T (2007) Arabidopsis WRKY70 is required for full RPP4-mediated disease resistance and basal defense against Hyaloperonospora parasitica. *Molecular Plant-Microbe Interaction*, 20(2): 120–128.

Knoth C, Salus MS, Girke T and Eulgem T (2009) The synthetic elicitor 3, 5-dichloroanthranilic acid induces NPR1-dependent and NPR1-independent mechanisms

of disease resistance in Arabidopsis. *Plant Physiology*, 150(1):.333-347.

Koch E, Meier BM, Eiben HG and Sluasarenko AJ (1992) A lipoxygenase from leaves of tomato (*Lycopersicon esculentum* Mill) is induced in response to plant pathogenic *Pseudomondas. Plant Physiology*, 99(2): 571–576.

Kogel KH and Langen G (2005) Induced disease resistance and gene expression in cereals. *Cell. Microbiology*, 7(11): 1555–1564.

Kuc J (2001) Concepts and direction of induced systemic resistance in plants and its application. *European Journal of Plant Pathology*. 107(1):7-12.

Kuc J (2006) "What's old and what's new in concepts of induced systemic resistance in plants and its application" *Multigenic and Induced Systemic Resistance In Plants*. Springer, US, 9–20.

Kunz W, Schurter R and Maetzke T (1997) The chemistry of benzothiadiazole plant activators. *Pesticide Science*, 50(4): 275–282.

Kunz W, Schurter R and Maetzke T (1997) The chemistry of Benzothiadiazole plant activators. *Pesticide Science*, 50(4):275-282.

Lawton K, Friedrich L, Hunt M, Weymann K, Delaney T, Kessman H, Staub T and Ryals J (1996) Benzothiadiazole induces disease resistance in Arabidopsis by activation of the systemic acquired resistance signal transduction pathway. *The Plant Journal*, 10(1): 71–82.

Lipinski CA, Lombardo F, Dominy BW and Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 64: 4–17.

Lupu R, Grossman S and Cohen Y (1980) The involvement of lipoxygenase and antioxidants in pathogenesis of powdery mildew on tobacco plants. *Physiology and Plant Pathology*, 16(2): 241-248.

Lynn DG and Chang M (1990) Phenolic signals in cohabitation: implications for plant development. *Annual Review of Plant Biology*, 41(1):497-526.

Lyon GD, Reglinski T and Newton AC (1995) Novel disease control compounds: the potential to immunize plants against infection. *Plant Pathology*, 44(3):407-427.

Manjunatha G, Roopa KS, Geetha NP and Shetty HS (2008) Chitosan enhances disease resistance in pearl millet against downy mildew caused by *Sclerospora graminicola* and defence-related enzyme activation. *Pest Management Science*, 64(12):1250-1257.

Musetti R, Vecchione A, Stringher L, Borselli S, Zulini L, Marzani C, D'Ambrosio M, di Toppi LS and Pertot I (2006) Inhibition of Sporulation and Ultrastructural Alterations of Grapevine Downy Mildew by the Endophytic Fungus *Alternaria alternata*. *Phytopathology*, 96 (7): 689-698.

Ohta H, Shuida K, Peng YL, Furusawa I, Shishiyama J, Aibara S and Morita Y (1991) A lipoxygenase pathway is activated in rice after infection with the rice blast fungus *Magnapoarthe grisea. Plant Physiology,* 97(1): 94–98.

Okubara PA, Schroeder KL and Paulitz TC (2005) Real-time polymerase chain reaction: applications to studies on soil-borne pathogens. *Canadian Journal of Plant Pathology*, 27(3):300-313.

Ostendorp M, Kunz W, Dietrich B and Staub T (2001) Induced disease resistance in plants by chemicals. *European Journal of Plant Pathology*, 107(1): 19-28.

Raj NS, Shetty NP and Shetty HS (2004) Proline-an inducer of resistance against pearl millet downy mildew disease caused by *Sclerospora graminicola*. *Phytoparasitica*, 32(5):523-7.

Ross AF (1966) Systemic effects of local lesion formation. *Viruses of plants,* 127-150.

Safeeulla KM (1976) Biology and Control of the Downy Mildews of Pearl Millet, Sorghum and Finger Millet.Wesley Press, Mysore, India, 304.

Sarosh BR, Sivaramakrishnan S and Shetty HS (2005) Elicitation of defense related enzymes and resistance by L-methionine in pearl millet against downy mildew disease caused by *Sclerospora graminicola*. *Plant Physiology and Biochemistry*, 43(8):808–815.

Shailasree S, Sarosh BR, Vasanthi NS and Shetty HS (2001) Seed treatment with betaaminobutyric acid protects Pennisetum glaucum systemically from Sclerospora graminicola. *Pest Management Science*, 57(8):721–728.

Sharathchandra RG, Niranjan RS, Shetty N P, Amruthesh KN and Shetty HS (2004) A chitosan formulation Elexa induces downy mildew disease resistance and growth promotion in pearl millet. *Crop Protection*, 23(10): 881-888.

Siedow JN (1991) Plant lipoxygenase: structure and function. *Annual Review of Plant Biology*, 42(1): 145-188.

Singh SD and Gopinath RA (1985) Seedling inoculation technique for detecting downy mildew resistance in pearl millet. *Plant Disease*, 69(7):582-584.

Smith-Becker J, Keen NT and Becker JO (2003) Acibenzolar-Smethyl induces resistance to Colletotrichum lagenarium and cucumber mosaic virus in cantaloupe. *Crop Protection*, 22(5): 769–774.

Sudisha J, Shigeru M, Amruthesh KN and Shetty HS (2007) Activity of cyazofamid against *Sclerospora graminicola,* a downy mildew disease of pearl millet. *Pest Management Science*, 63(7):722–727.

Vallad GE and Goodman RM (2004) Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science*, 44(6):1920-1934.

Wafaa MH and Haggag M (2002) Sustainable agriculture management of plant diseases. *Online Journal of Biological Science*, 2(4):280–284.

Walters D, Walsh D, Newton A and Lyon G (2005) Induced resistance for plant disease control: Maximizing the efficacy of resistance elicitors. *Phytopathology*, 95(12): 1368-1373.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Diversity of benthic macro-invertebrates in four tributaries of River Narmada in the central zone, India

Golwalkar Rachna Deo[1], Sleeqa Banoo[1], Mustaq Tehmeena[1], Diwan Suniti[1], Kumar Ankit[2] and Vyas Vipin[3]*

[1]Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal, India
[2]Department of Environmental Sciences and Limnology, Barkatullah University, Bhopal, India
[3]Department of Bioscience, Barkatullah University, Bhopal, India
*Corresponding Author- vyasvipin992@gmail.com

Manuscript details:

Received: 03.02.2016
Accepted: 16.03.2016
Published : 30.03.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Golwalkar Rachna Deo, Sleeqa Banoo, Mustaq Tehmeena, Diwan Suniti, Kumar Ankit and Vyas Vipin (2016) Diversity of benthic macro-invertebrates in four tributaries of River Narmada in the central zone, India. *International J. of Life Sciences*, 4(1): 107-115.

Acknowledgement
Authors would like to thanks Miss Anjali Chaurasiya, Mr. Vikram Singh and Mr. Bilal Nabi for their help and support during field visits. Sincere thanks to Mrs. Reetu Sharma who helped during identification of benthic macro-invertebrates.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

The present study examines the distribution of benthic macro-invertebrate fauna in the four seasonal tributaries of River Narmada in the central zone to evaluate the benthic macro-invertebrates community assemblages in predicting the water quality status. During the present investigation, total 8 sampling stations (two sampling stations on each tributary) were identified and from these stations 30 taxa of benthic macro-invertebrates were recorded. Among the major taxonomic composition phylum arthropoda was found at dominant (63%) position, whereas values of Shannon-Wiener diversity index was found between 1.12 – 2.10 which indicates moderate pollution status at all stations. Values of Pielou evenness index (0.67-0.96) showed equability in the apportionment of individuals among the species at all stations while, range of Margalef diversity index varied from 0.94 to 3.58 indicates extremely low species richness and low abundance with physically disturbed areas in poor condition of colonization by aquatic organisms.

Keywords- Benthic-macroinvertebrate, Tributary, Central Zone, River Narmada

INTRODUCTION

The benthic macro-invertebrates community of the lotic ecosystem, like other communities has a series of attributes that do not reside in its individual species components and have meaning only with reference to the community level of integration such as species diversity, growth in the form and structure, dominance, relative abundance and trophic structure. One of these attributes many of these or all, depending upon situation may be changed with the changing ecology of the water body concerned. Species are distributed individualistically according to their own genetic characteristics and population of most of the species tends to change gradually along the environmental gradients. Most species are not in obligatory associations with other species, which suggests that

association is formed with many combinations of species, and vary continuously in space and time. Hence, a study of benthic macro-invertebrates community composition and dynamics of different population of the community becomes a reliable source to provide the picture of environmental status and influence of changing limnology of the water body concerned (Bhandarkar and Bhandarkar, 2013).

Benthic macro-invertebrates perform a variety of functions in freshwater ecosystem. They have an important influence on nutrient cycle, primary productivity, decomposition and translocation of material (Wallace and Webster, 1996; Covich *et al.,* 1999). They are the most commonly used for bio-monitoring in lotic habitat worldwide (Bonada *et al.,* 2006). They play an important role in the mineralization and recycling of organic matter and are an important tool for improving and preserving water quality (Bilgrami and Munshi, 1985; Venkateswarlu, 1986). Alteration produced in the physical and chemical status of the riverine ecosystem becomes recognizable through elasticity of the community structure of the organisms (Wilhm and Dorris, 1968; Cairns and Dickson, 1971).

The aim of the present study was to measure the diversity of benthic macro-invertebrates in the four seasonal tributaries of River Narmada in the central zone. The Shannon index of species diversity of benthic macro-invertebrate in fact summarizes physico-chemical and hydrobiological information in a significant manner, condensing it in a single index.

MATERIALS AND METHODS

Study area

River Narmada is the fifth largest river of India covers central part of our country and originates from Amarkantak (Madhya Pradesh) flows into west direction to meet with the Gulf of Cambay in the Arabian Sea (Gujarat). The study area is located in the central zone of River Narmada which lies in Sehore district of Madhya Pradesh (Figure- 1). Here four seasonal tributaries join the main river from right bank and these tributaries were considered for the investigation. Base map of these tributaries is shown in Figure- 2. Details of four tributaries are mentioned below:

A. Chandni Nalla-

This seasonal tributary is located in the central zone of River Narmada between Shahganj and Jahanpur villages of Sehore and Raisen districts of Madhya Pradesh. This area has its own natural landscape and lies on the foot hills of Vindhyan mountain range. The total length of this nalla from its origin upto the confluence with River Narmada is 29.35 kms whereas, major basin area falls under agricultural land use and very little area falls under forest land cover.

B. Gadaria Nalla-

This is second seasonal tributary originates from Vindhyan hilly ranges. Maximum watershed of this nalla falls under dense forest land cover only few areas in dominated with agricultural land use near confluence with River Narmada. It joins the river from right bank between Jamuniya and Budhni Ghat villages. The total length of this nalla from its origin upto the confluence with River Narmada is 34.58 kms. Basin of this nalla falls in Sehore and Raisen districts of Madhya Pradesh.

C. Kaliyadeh Stream-

The third tributary which is considered for the study was Kaliyadeh stream. It originates from the Vindhyan range and converge with River Narmada between Mou Kalan and Holipura villages from right bank after covering a distance of 27.70 kms. This is a seasonal stream and two major industries have been established namely Vardhman and Trident inside its watershed area. Maximum watershed area is covered through dense forest vegetation and very little area is used for agricultural practice. Basin of this tributary lies in Sehore and Raisen districts of Madhya Pradesh.

D. Bhagner Stream-

This is the fourth and last seasonal tributary in the study area which joins the river in the central region. Basin of this tributary lies in Sehore district of Madhya Pradesh. Total length of this stream is 33.79 km from origin upto the confluence. Its watershed area initially lies under forest cover of Vindhyan range but as it travels to meet the river from right bank due to excessive human intervention forest cover has been changed into agricultural land use.

Sampling stations

During the present investigation, two samplings were conducted on each tributary from two identified sampling stations. Geographical locations of identified sampling stations are given in Table- 1. Two sampling

stations were chosen per tributary because of access and approach as well as good connection with road. Locations of sampling stations chosen for the study are shown in Figure- 3.

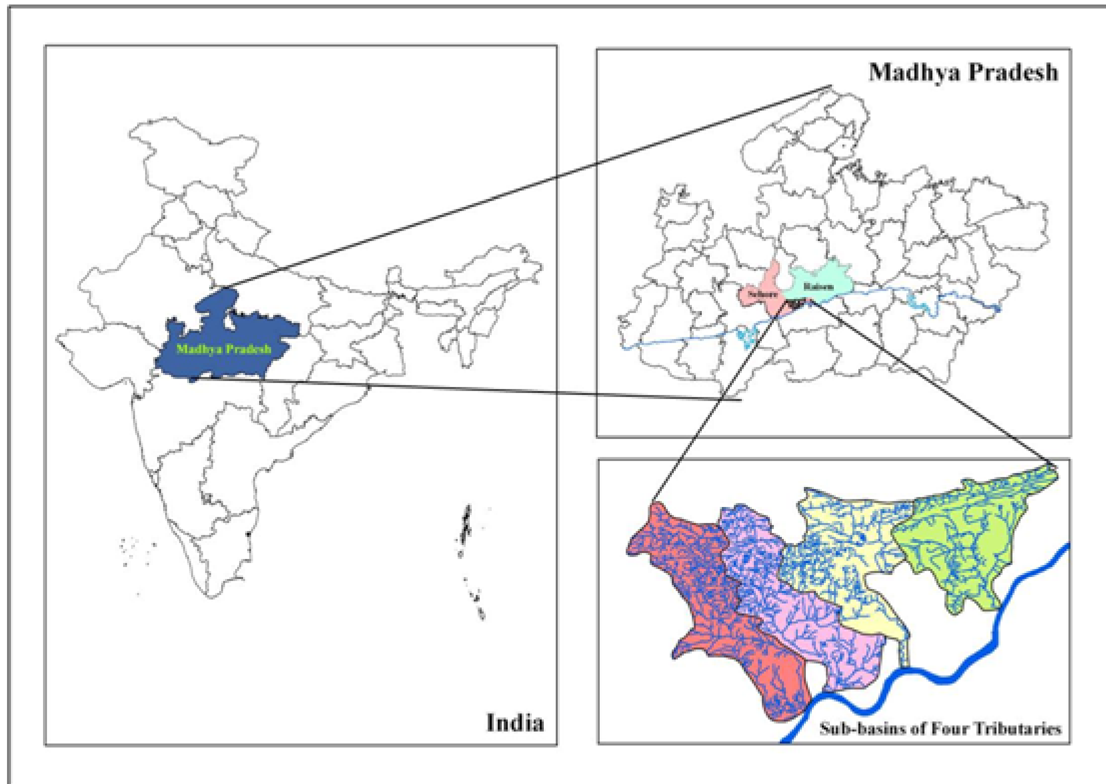


Figure- 1: Location map of the study area

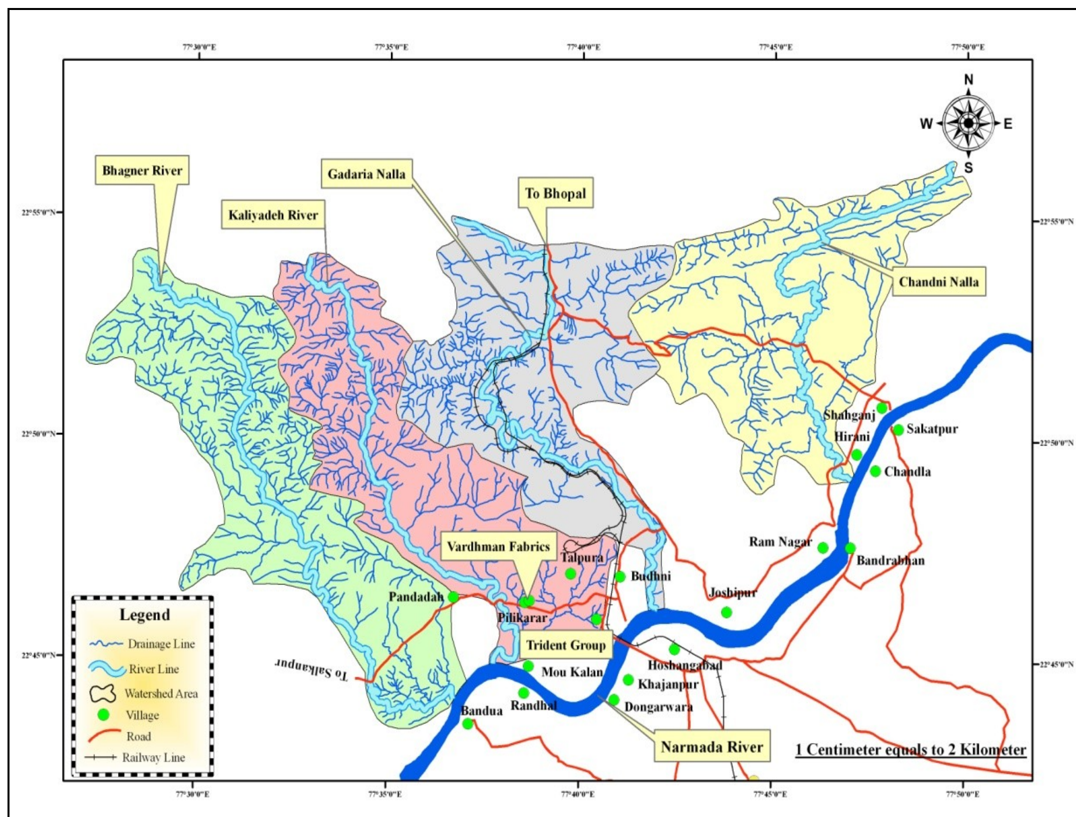


Figure- 2: Base map of the study area

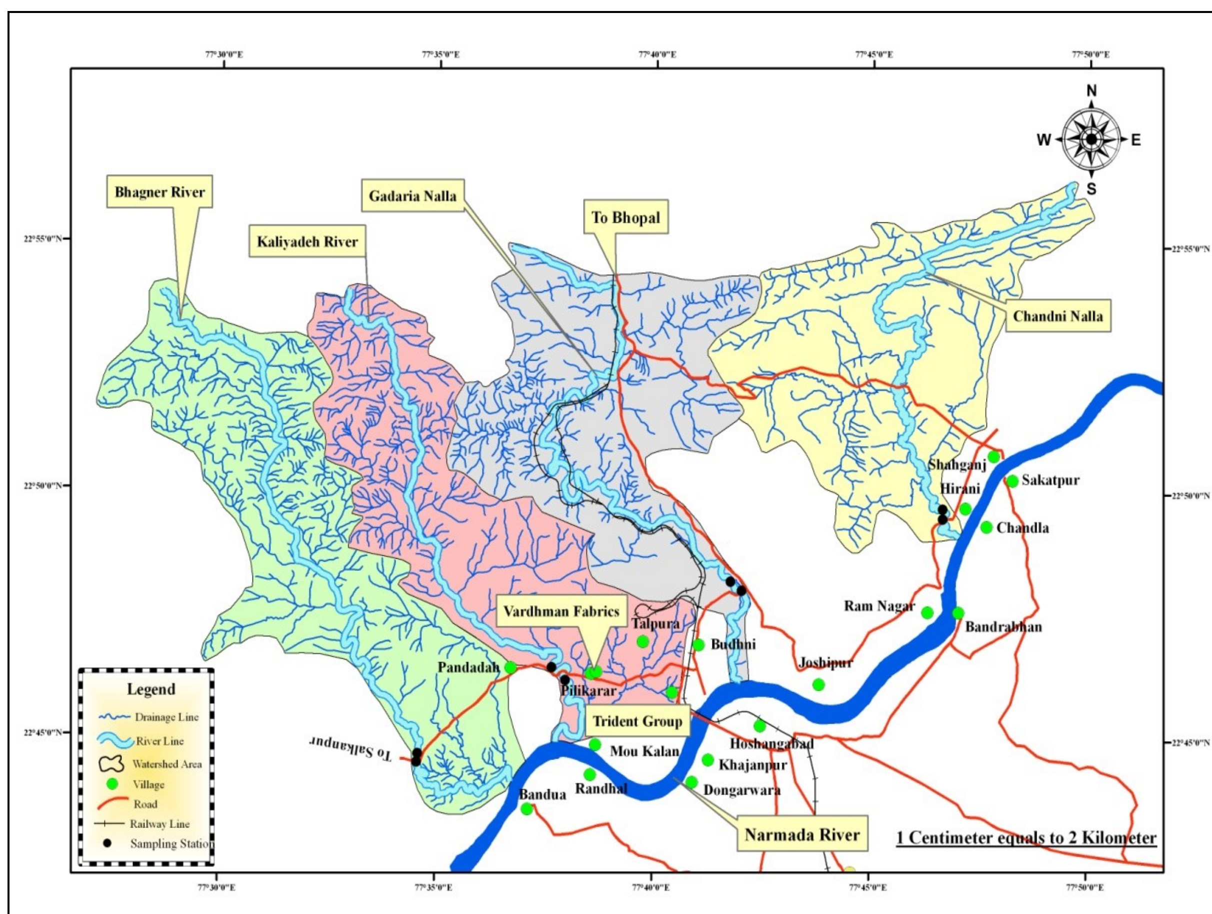


Figure- 3: Sampling stations of the study area

Table- 1: Geographical location of sampling stations

S. No.	Stream name	Sampling station	Station code	Longitude	Latitude
1	Chandni Nalla	First	CD- 1	77º46'40.86" E	22º49'30.43" N
		Second	CD- 2	77º46'43.45" E	22º49'35.68" N
2	Gadaria Nalla	First	GD- 1	77º42'2.7" E	22º48'0.7" N
		Second	GD- 2	77º41'47.01" E	22º48'11.29" N
3	Kaliyadeh Stream	First	KL- 1	77º37’ 45.91” E	22º46’ 24.17” N
		Second	KL- 2	77º37’ 54.88” E	22º46’ 20.81” N
4	Bhagner Stream	First	BH- 1	77º34′27.30″ E	22º44′29.78″ N
		Second	BH- 2	77º34′30.13″ E	22º43′31.31″ N

Collection, Sieving, Sorting, Preservation, Transportation and Identification of samples

Most of the sampling stations fall under shallow zone with macrophytic vegetation. So, for the collection of benthic macroinvertebrates D- frame net was used and some were collected directly from stones using forceps and brushes very carefully. Samples were sieved using 0.3 micron mesh size brass sieve and with utmost care these were sorted and kept safely in reagent grade wide mouth plastic screw bottle with 4% solution used as preservative to transport the sample carefully to the laboratory for further work.

In next step, identification of macroinvertebrates was done with the help of stereo microscope and hand lens with 6x zoom capacity to observe the finest details about the organisms. Available keys and monographs were used to identify fauna upto their lower taxonomic levels (Needham and Needham, 1962; Rao, 1989; McCafferty and Provonsha, 1998; Dey, 2007).

Statistical Analysis

Diversity of benthic macroinvertebrates was statistically treated with the help of Shannon-Wiener's diversity index (H'), Pielou's evenness index (J') and Margalef diversity index (d).

Shannon-Wiener's diversity index (H')

$$H' = -\Sigma [(n_i / N)*(\text{Log } N (n_i) / N)]$$

H': Shannon-Wiener Diversity Index
n_i: Number of individuals belonging to i species
N: Total number of individuals
LN: Natural Log base N of the number

Pielou's evenness index (J')

$$J' = H'/H'_{max}$$

J': Pielou evenness index
H': The observed value of Shannon index
H'_{max}: LN(S)
LN: Natural Log base N of the number
S: Total number of species

Margalef diversity index (d)

$$d = (S - 1) / \text{LogN}(N)$$

d = Margalef Diversity Index
S = Total number of species
N = Total number of individuals
LN: Natural Log base N of the number

RESULTS AND DISCUSSION

A total of 30 taxa were found from eight sampling stations which belong to two phylum i.e. Mollusca and Arthropoda (Table- 2).

Phylum mollusca was represented by 2 classes i.e. Gastropoda and Bivalvia. Class gastropoda was represented by 2 orders, 4 families and 7 genera, whereas class Bivalvia was also represented by 2 orders, 3 families and 4 genera. Phylum arthropoda was represented by 3 classes i.e. Insecta, Crustacea and Arachnida. Class insecta was represented by 5 orders, 13 families and 17 genera, while class crustacea was represented by 1 order, 1 family and 1 genus, whereas class arachnida was represented by 1 order, 1 family and 1 genus.

In the present investigation, phylum arthropoda was found in dominant position with 63% followed by phylum mollusca with 37% occupancy in total faunal assemblage (Figure- 4). Dominance of arthropoda was also reported in River Narmada (Kumar and Vyas, 2014), in Morand River a sub-tributary of River Narmada (Sharma *et al.,* 2013), in Kalsi Dehradun segment of River Yamuna (Ishaq and Khan, 2013), in Ken River (Nautiyal and Mishra, 2013) and near water intake point in River Narmada (Vyas *et al.,* 2012).

In statistical procedure, values of Shannon-Wiener's diversity index ranged between 1.12 and 2.10 (Figure- 5). Minimum value of index was observed at station BR- 1 while maximum value was found at station GD- 1. Shannon is a sensitive indicator of pollution and its values do not fluctuated widely. This is an index applied to biological systems, by derived from a mathematical formula used in communication area by Shannon in 1948 (Mandaville, 2002). This is most preferred index among the other diversity indices and values are between 0.0 – 5.0. Results are generally in 1.5 - 3.5 and it exceeds 4.5 very rarely. In the present study, range of this index was recorded from 1.12 to 2.10 which indicate moderate pollution with altered habitat structure. Khan *et al.,* (2007) showed the diversity index ranging from 1.20 to 1.49 in their study on Mouri River, Bangladesh and Anbalagan *et al.,* (2004) observed values ranged 1.883 to 2.493 from 4 sampling stations of courtallam hills of Western Ghats. Wilhm and Dorris, (1966) proposed a relationship between species diversity and pollution status of sampling sites as; species diversity value greater than 3.0 is clean water, values in the range of 1.0-3.0 indicate moderate pollution and values less than 1.0 indicate heavy pollution. According to them, all the selected sampling stations fall under moderate pollution.

Pielou's evenness index values ranged from 0.67 to 0.96 (Figure- 6) and minimum was recorded at station KL- 2 whereas, maximum was observed at CN- 2. Another major component of species diversity is evenness which is originated from Shannon diversity index, proposed by Pielou (1966). Evenness denotes a balanced relation between species and individual richness of a sample. This numerical digit (0 to 1) expresses the absolute distribution of relative abundance of species at specific site. In the present investigation, range of evenness index varied from 0.67 to 0.96 showed equitability in the apportionment

of individuals among the species at all stations. Higher values of this index indicate a low concentration of dominance of species diversity at a specific site (Mukherji and Nandi, 2004).

Table- 2: List of benthic macro-invertebrates recorded during the study

S. No.	Taxa	Tributaries							
		Chandni Nalla		Gadaria Nalla		Kaliyadeh River		Bhagner River	
		Sampling Stations							
		1	2	1	2	1	2	1	2
Phylum	**Mollusca**								
Class	**Gastropoda**								
Order	**Mesogastropoda**								
Family	**Viviparidae**								
1	Bellamya *bengalensis*	-	-	+	+	+	+	+	+
Family	**Thiaridae**								
2	Thiara (Melanoides) *tuberculata* (Muller)	+	+	+	+	+	+	-	-
3	Tarebia *lineata* (Gray)	+	-	+	+	-	+	+	-
4	Tarebia *granifera* (Lamarck)	+	-	+	+	-	+	-	-
Order	**Basommatophora**								
Family	**Lymnaeidae**								
5	Lymnaea (Pseudusuccinea) *acuminata* (Lamarck)	-	-	-	-	+	-	-	-
Family	**Planorbidae**								
6	Gyraulus *convexiusculus* (Hutton)	-	-	+	-	-	+	-	-
7	Indoplanorbis *exustus* (Deshayes)	-	-	-	-	-	-	-	+
Class	**Bivalvia**								
Order	**Trigoinoida**								
Family	**Amblemidae**								
8	Parreysia (Radiatula) *occata* (Lea)	+	-	+	+	+	+	+	+
9	Parreysia *corrugata*	-	-	+	+	-	-	-	-
Family	**Unionidae**								
10	Lamellidens *marginalis*	-	-	+	+	-	-	-	-
Order	**Veneroida**								
Family	**Corbiculidae**								
11	Corbicula *striatella* (Deshayes)	-	-	+	-	-	-	-	-
Phylum	**Arthropoda**								
Class	**Insecta**								
Order	**Diptera**								
Family	**Tabanidae**								
12	Tabanus *sps.*	-	-	-	-	+	-	-	-
Family	**Syrphidae**								
13	Eristalis *sps.*	-	-	+	+	-	-	+	+
Family	**Culicidae**								
14	Culex *sps.*	+	-	+	-	-	-	-	-

Table- 2: Continued…

S. No.	Taxa	Tributaries							
		Chandni Nalla		Gadaria Nalla		Kaliyadeh River		Bhagner River	
		Sampling Stations							
		1	2	1	2	1	2	1	2
Order	**Odonata**								
Family	**Gomphidae**								
15	Gomphus *sps.*	+	+	+	-	+	+	-	+
16	Octogomphus *sps*	-	+	-	-	-	-	-	-
17	Aphylla *sps.*	-	-	-	+	-	-	-	-
Family	**Cordulegastridae**								
18	Cordulegaster *sps.*	+	+	-	-	+	+	-	-
Family	**Coenagrionidae**								
19	Enallagma *sps.*	-	-	+	-	-	-	-	-
Order	**Hemiptera**								
Family	**Nepidae**								
20	Nepa *sps.*	-	-	-	+	+	-	-	-
21	Ranatra *sps.*	-	-	-	+	-	-	-	-
Family	**Corixidae**								
22	Sigara *Sps.*	-	-	-	-	+	+	-	-
Family	**Belestomatidae**								
23	Belostoma *sps.*	-	-	-	+	-	-	-	-
Order	**Ephemeroptera**								
Family	**Heptageniidae**								
24	Heptagenia *sps.*	-	-	-	-	-	+	-	-
Order	**Coleoptera**								
Family	**Haliplidae**								
25	Peltodytus *sps.*	-	-	-	+	+	-	-	-
Family	**Gyrinidae**								
26	Dineutus *sps.*	+	-	-	+	+	-	-	-
27	Gyrinus *sps*	+	+	-	-	-	-	-	-
Family	**Hydrophilidae**								
28	Berosus *sps.*	+	-	-	-	+	+	-	-
Class	**Crustacea**								
Order	**Decapoda**								
Family	**Palaemonidae**								
29	Palaemon *sps.*	+	-	-	+	+	+	-	-
Class	**Arachnida**								
Order	**Araneae**								
Family	**Pisauridae**								
30	Dolomedes *sps.*	-	-	-	-	-	+	-	-
Total		**11**	**5**	**13**	**15**	**13**	**13**	**4**	**5**

Table- 3: Values of Statistical Indices

Statistical Indices	Tributaries							
	Chandni Nalla		Gadaria Nalla		Kaliyadeh River		Bhagner River	
	Sampling Stations							
	CN- 1	CN- 2	GD- 1	GD- 2	KL- 1	KL- 2	BR- 1	BR- 2
Shannon Index (H')	2.06	1.55	2.10	1.91	1.92	1.72	1.12	1.14
Pielou Index (J')	0.86	0.96	0.82	0.71	0.75	0.67	0.81	0.71
Margalef Index (d)	2.45	2.06	2.97	3.58	3.15	2.87	0.94	1.20

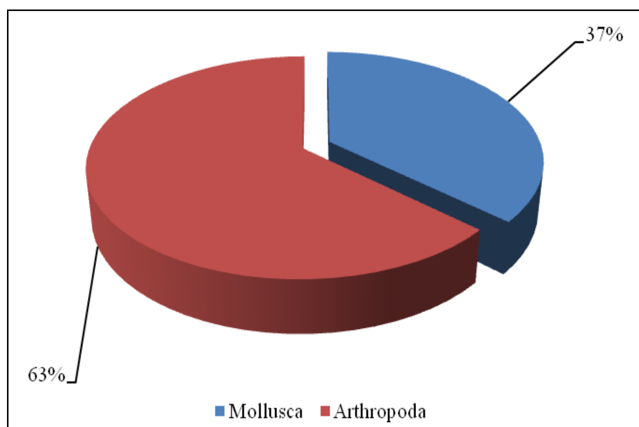


Fig. 4: Percent composition of major taxonomic groups

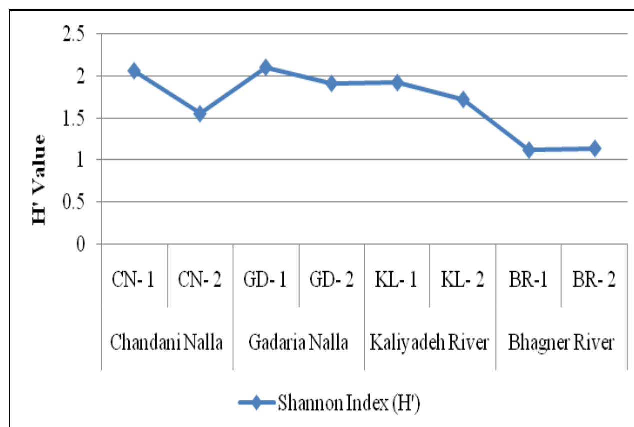


Fig. 5: Shannon-Wiener diversity index values at different stations

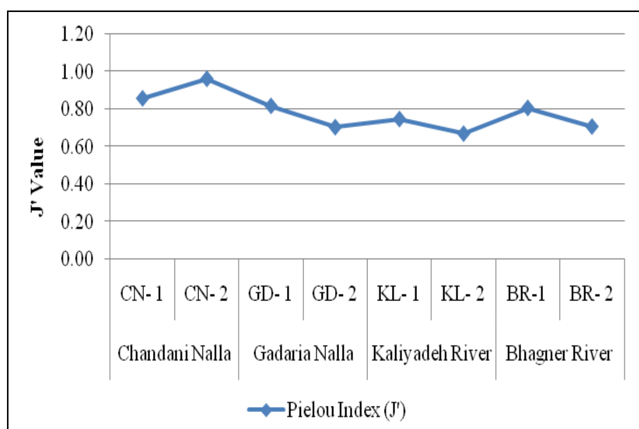


Fig.6: Pielou's evenness index values at different stations

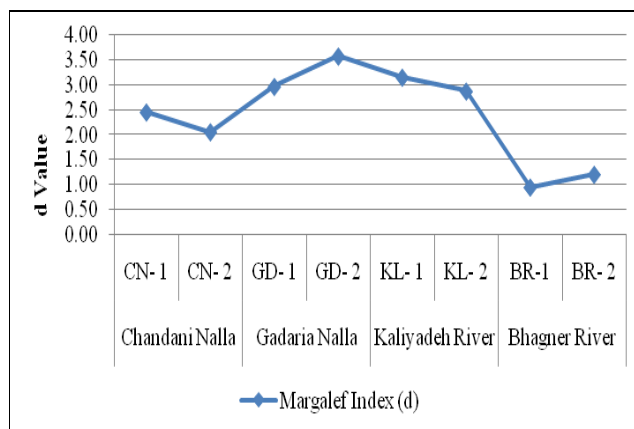


Fig. 7:Margalef diversity index values at different stations

The values of Margalef diversity index varied between 0.94 and 3.58 (Figure- 7). Minimum range of index was found at station BR- 1 while maximum was recorded at station GD- 2. Margalef diversity index which is a species richness index is expressed by simple ratio between total species and total number (or importance value N), the Margalef's diversity index are proposed by Margalef (1958), larger the index value the more health the body of water, when it tend towards 1.0, pollution is thought to increase and damage should be suspected. According to Margalef (1956) the higher diversity values reflect the suitability of habitat for the organism in one hand while on the other the high species diversity has been reported to be correlated with longer food chain and complex food web of the ecosystems and also more stable community. It has no limit value and it shows a variation depending upon the number of species. During the present investigation, range of this index varied from 0.94 to 3.58. Low index value was

observed during the present study indicates extremely low species richness and low abundance with physically disturbed areas in poor condition of colonization by aquatic organism (Bhandarkar and Bhandarkar, 2013).

REFERENCES

Anbalagan S, Kaleeswaran B and Balasubramanian C (2004) Diversity and trophic categorization of aquatic insects of courtallam hills of Western ghats. *Entomon,* 29(3): 215-220.

Bhandarkar, SV and Bhandarkar, WR (2013) A study on species diversity of benthic macro invertebrates in freshwater lotic ecosystems in Gadchiroli district Maharashtra. *Int. J. of Life Sciences,* 1(1): 22-31.

Bilgrami, KS and Dutta Munshi, JS (1985) Ecology of River Ganges: Impact on human activities and conservation of aquatic biodata (Patna to Farakka), May-1982- April-1985, Allied Press, Bhagalpur, India.

Bonada N, Prat N, Resh VH and Statzner B (2006) Development in aquatic insect Bio-monitoring: a comparative analysis of recent approaches. *Annual Review of Entomology,* 51: 495-523.

Cairns JR and Dickson KL (1971) A simple method for the biological assessment of the effects of waste discharges on aquatic bottom dwelling organisms. *J. Wat. Pollut. Control.* Feb., 43: 755-772.

Covich AP, Palmer MA and Crowl TA (1999) The role of benthic invertebrate species in freshwater ecosystems: Zoobenthic species influence energy flows and nutrient cycling. *BioScience,* 49 (2): 119-127.

Dey, RA (2007) Handbook on Indian freshwater molluscs. Zoological Survey of India, Kolkata, pp: 399.

Ishaq, F and Khan, A (2013) Diversity pattern of macrozoobenthos and their relation with qualitative characteristics of River Yamuna in Doon Valley, Uttarakhand. *American-Eurasian Journal of Toxicological Sciences,* 5(1): 20-29. DOI:10.5829/idosi.qests.2013.5.1.72125.

Khan AN, Kamal D, Mahmud MM, Rahman MA and Hossain MA (2007) Diversity, distribution and Abundance of Benthos in Mouri River, Khulna, Bangladesh. *Int. J. Sustain Crop Prod,* 2(5): 19-23.

Kumar A and Vyas V (2014) Diversity of macrozoobenthos in the selected reach of River Narmada (Central Zone), India. *International Journal of Research in Biological Sciences*. 4(3): 60-68.

Mandaville SM (2002) Benthic macro invertebrates in freshwater_taxa tolerance values, metrics, and protocols, project H-I. (Nova Scotia: Soil & Water Conservation Society of Metro Halifax).

Margalef R (1956) Information Y diversidad especificaenlas communidades de organisms. *Invest. Pesg,* 3: 99-106.

McCafferty WP and Provonsha AV (1998) The fishermen's and ecologist illustrated guide to insects and their relatives: Aquatic Entomology. Jones and Bartlett Publishers, pp: 448.

Mukherji M and Nandi NC (2004) Studies on macrozoobenthos of Rabindra sarovar and Subhas sarovar in Kolkata in relation to water and sediment characteristics. *Res. Zool. Surv. India.* Occ. Paper No. 225: 1-119.

Nautiyal P and Mishra AS (2013) Variation in benthic macro invertebrate fauna as indicator of land use in the Ken River, Central India. *Journal of Threatened Taxa,* 5(7): 4096-4105.

Needham JG and Needham PR (1962) A Guide to the study of Freshwater Biology. Holden- Day, Inc., San Francisco, pp: 108.

Pielou EC (1966) The measurement of diversity in different types of biological collections. *J. Theoret. Bioi.,* 13: 131-144.

Sharma R, Kumar A and Vyas V (2013) Diversity of macrozoobenthos in Morand River- A tributary of Ganjal River in Narmada Basin. *International Journal of Advanced Fisheries and Aquatic Science,* 1(1): 57-65.

Subba Rao NV (1989) Handbook freshwater molluscs of India. Zoological Survey of India, pp: 289.

Venkateswarlu V (1986) Ecological studies on the rivers of Andhra Pradesh with special reference to water quality and Pollution. *Proc. Indian Sci. Acad.,* 96(6): 495-508.

Vyas V, Bharose S, Yousuf S and Kumar A (2012) Distribution of Macrozoobenthos in River Narmada near water Intake point in India. *Journal of Natural Sciences Research,* 2(3): 18-24.

Wallance JB and Webster JR (1996) The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology,* 41: 115-139.

Wilhm JL and Dorris TC (1966) Species diversity of benthic macro-invertebrates in a stream receiving domestic and oil refinery effluents. *Am Midl. Nat.,* 76: 427-449.

Wilhm RL and Dorris TC (1968) The biological parameters for water quality criteria. *Bio Science,* 18: 477-492.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Ground water quality assessment and its impact with special reference to Chhindwara District of Madhya Pradesh, India

Neelima Bagde

Department of Zoology, R.S. Govt. Girls College, Chhindwara, M.P., India.
E-mail: neelimabagde@gmail.com

Manuscript details:

Received: 05.01.2016
Accepted: 16.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Bagde Neelima (2016) Ground Water Quality Assessment and its Impact with Special Reference to Chhindwara District of Madhya Pradesh, India the broiler farms in Madhya Pradesh. *International J. of Life Sciences*, 4(1): 116-120.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

The Earth is full of natural resource needed for the development of mankind. The day by day increased demand has developed new methods of water quality assessment and management. The study was carried out for the ground water quality assessment of 9 Tahsil/Blocks of Chhindwara district. Water is the basic resource for sustaining all human activities, so its provision in desire quantity and quality is most important. For assessment of water quality of the district were selected Junnardeo, Chhindwara, Chourai, Tamia and Pandhurna. Samples of ground water were collected from 5 blocks of the affected area and analyzed for physico chemical parameters like pH, Electrical conductivity, alkalinity, total hardness, fluoride ion were analyzed. Some water sample show higher fluoride ion concentration, higher turbidity. Over all some parts of bore well/tube well water and hand pump water needed treatment for drinking purpose due to hardness and fluoride are present in desirable limit. The present study is focused on measuring the quality of ground water in rural areas of Chhindwara district and its effect on human health. The sample were found to have high pH, indicating alkalinity, high fluoride concentration is mainly found in Tamia, Chhindwara, Jamai, Pandhurna and Chaurai on the contrary these findings, majority of people living in these areas were suffering from fluoride related disease, so the study argues about the need of proper analysis and importance of water treatment and management system in current times

Keywords: Water quality, Fluorides, Diseases, Assessment, Parameters.

INTRODUCTION

Water is an essential resource for all life on the planet. Water covers over 71% of the earth's surface and is a very important natural resource for people. Only three percent of it is fresh and two-thirds of the fresh water locked up in ice caps and glaciers. It is the fundamental right of every individual to get pollution free water, Juneja and Chaudhary (2013). Water plays an essential role in human life. Although statistics, the WHO reports that approximately 36% of urban and 65% of rural Indian were

without access to safe drinking water. Fresh water is one of the most important resources crucial for the survival of all the living beings. It is even more important for the human being as they depend upon it for food production, industrial and waste disposal, as well as cultural requirement. Human and ecological use of ground water depends upon ambient water quality. Human alteration of the landscape has an extensive influence on watershed hydrology. Ground water plugs a vital role in human life. The consequences of urbanization and industrialization leads to spoil the water for agricultural purposes ground water is explored in rural especially in those areas where other sources of water like dam and river or a canal is not considerable. During last decade, this is observed that ground water get polluted drastically because of increased human activities. Consequently number of cases of water borne diseases has been seen which a cause of health hazards, Neeraja et al. (2012).

The major part of the district is occupied by Deccan Trap. The coal bearing places are lower Gondwanas in parts of Jamai, Jamai blocks and Parasia. There are Pench and Kanhan rivers which flow in this trap. Ground water occurs under phreatic and semi-confined to confined condition. The entire district, command and Non Command areas, falls under safe Category, where stage of ground water development is 89.42%. The net annual ground water availability in the district is 1101.50 MCM and draft from all uses is 558.96 MCM, Rao (2001, 2002, 2003).

Chhindwara district is located on the Southwest region of 'Satpura Range of Mountains'. The district is spread over an area of 11,850 Sq. km and is located at the southern boundary of the state, laying between North Latitudes 21^{0} 28’ and 22^{0} 50’ and East longitudes 78^{0} 15’ and 79^{0} 25’ falls under the Survey of India Topo Sheet No. 55 J, K, N, & O. The district is bounded by Narsinghpur and Hoshangabad district in the north, Seoni district in the east, Betul district in the west and by Maharashtra state in the south, DGWB (2007).

Study Area

The District is divided into 9 Tahsils/Blocks (Chhindwara, Tamia, Parasia, Jamai, Chourai, Amarwara, Sausar, Bichhua and Pandhurna). There are 1984 villages in the district, out of which 1906 villages are habitated.

Map 1:Map of Chhindwara District

As per Census 2011, the total population of the district is 20, 90, 922 out of which 76.90% belong to rural areas. The Scheduled Caste and Scheduled Tribes population is 2, 14,201 and 6, 41,421 respectively.

The district lies in parts of the Narmada and the Godavari basin, Wainganga sub basins. The total catchments areas of the Narmada & the Wainganga rivers falling in the district are 3,555 and 8,295 Sq. km respectively. The major tributaries of the Godavari River are Kanhan, Pench and Wardha, while Sakkar, Sitarewa, Dudh are tributaries of Narmada River

MATERIALS AND METHODS

In present investigation that fifty water samples ie ten from each blocks from five blocks were collected. For assessment of water quality of the Kanan valley coal mines 10 villages were selected area of the affected area. Samples of ground water were collected from 10-10 villages of each block. These samples of ground water were chemically analyzed. The studies were carried out in the affected area. Ground water is the main source for drinking water supply both in rural and urban areas and is supplementing the surface water supply schemes. A total number of 10,326 shallow tube wells fitted with hand pumps are operational to provide drinking water to as many as 4482 inhabited villages, (PHED Chhndwara).

Parameters

Physico chemical parameters like pH, Electrical Conductivity, Salinity, Total Hardness, Fluoride ion, Iron and Turbidity were analyzed by help of PHE Department Chhindwara.

RESULTS AND DISCUSSION

The result of the chemical analysis of water in the present study in Table-1,2,3,4,5 so it is necessary to make a comparison of water given by WHO standards. The pH of water shows variation in its ranges. It indicates that they are in range of water quality parameter permissible limits. The pH value of water samples of 35 stations ranges between 7.35 and 7.93 values were of alkaline in nature and 15 stations values were of acidic in nature. The EC of water samples shows wide variation in all five blocks. The electrical conductivity (EC) values were found to be in the range of 332 and 1722 µS/cm. The EC values exceeding limit, 1000 µS/cm, were noticed at three villages, Viz., Chhindi (1005 µS/cm), Silwani (highest 1722 µS/cm) and Chhindwara (1132 µS/cm). The high EC values of these villages may be attributed to the primary minerals of earth's crust dissolved in water samples. The concentration of fluoride ion is higher in deeper levels and appears increasing with depth and also from recharge areas to discharge areas as revealed by the fact that the number of villages with high fluoride concentration is located on either side of Pench River. The high value of fluoride ion, 19.90 mg/l, has been recorded in deep ground water in at Rajakho village. Occurrence of high fluoride is mainly found in parts of Jamai, Chhindwara, Chourai, Bichhua and Pandhurna blocks. It is also noticed that the comparatively high fluoride concentration is found in the hand pumps located near and intersection of lineaments e.g. Sevajpani village (13.7 mg/l), Maduadhana(16.5 mg/l), Khatkar(10.4 mg/l), Hivarkhedi(15.75 mg/l) Ghat Parasia, (13.00 mg/l) etc.

Table-1: Ground water analysis of different villages of Junnardeo block

Sr. No.	Name of village	Volume	Source	Fluoride 1.00/1.50	Salinity 200/1000	Iron 0.10/1.00	Turbidity 2.50/10.00	pH 6.50/8.60	EC	Hardness 200/600
1	Panara	100ml each	Hand pump	8.00	578.82	1.76	10.03	7.9	432	644
2	Dungariya	" "	" "	1.24	278.69	0.82	20.41	7.9	334	392
3	Jamba kiradi	" "	" "	3.90	121.48	0.43	19.02	7.7	765	376
4	Chikhalmau	" "	" "	1.78	1357.74	0.51	7.24	8.5	1000	588
5	Moyari	" "	" "	1.28	1350.06	0.92	10.20	10.5	905	136
6	Karan pipariya	" "	" "	1.37	39.29	0.73	8.96	9.6	607	32
7	Gorakhghat	" "	" "	3.40	164.35	0.46	13.96	7.8	343	284
8	Kohliya	" "	" "	2.80	282.26	0.56	26.83	8.1	532	536
9	Purainakhalsa	" "	" "	1.01	364.44	0.64	13.24	7.9	389	756
10	Pipariya manu	" "	" "	3.20	150.06	0.21	7.12	7.5	865	452

Source- P.H.E. Department Chhindwara.

Table 2: Ground water analysis of different villages of Tamia block

Sr. No.	Name of village	Volume	Source	Fluoride 1.00/1.50	Salinity 200/1000	Iron 0.10/1.00	Turbidity 2.50/10.00	pH 6.50/8.60	EC	Hardness 200/600
1	Tamia	100ml each	Hand pump	0.38	778.82	.39	12.00	7.83	709	544
2	Chhindi	" "	" "	1.48	378.69	0.92	20.41	7.84	005	492
3	Delakhari	" "	" "	0.90	221.48	0.53	19.02	7.8	777	306
4	Lahgudna	" "	" "	1.78	357.74	0.41	7.24	7.86	779	528
5	Patalcot	" "	" "	1.48	550.06	0.82	10.20	8.5	1009	236
6	Rated	" "	" "	1.87	639.29	0.93	8.96	9.6	345	320
7	Jatachapar	" "	" "	1.37	347.0	0.64	11.7	11.2	845	586
8	Gorakhnath	" "	" "	3.90	1164.35	0.46	13.96	7.8	756	384
9	Kolya	" "	" "	2.40	882.26	0.56	26.83	8.1	365	236
10	Sangakheda	" "	" "	0.65	414.38	0.72	13.37	7.8	567	396

Source- P.H.E. Department Chhindwara.

Table 3: Ground water analysis of different villages of Chhindwara block

Sr. No.	Name of village	Volume	Source	Fluoride 1.00/1.50	Salinity 200/1000	Iron 0.10/1.00	Turbidity 2.50/10.00	pH 6.50/8.60	EC	Hardness 200/600
1	Chhindwara	100ml each	Hand pump	0.30	678.82	1.39	10.33	7.38	1132	745
2	Ghatparasia	" "	" "	1.58	478.69	0.82	9.41	7.84	1006	392
3	Khajri	" "	" "	4.10	229.48	0.43	10.02	7.8	877	376
4	Rajakho	" "	" "	19.90	857.74	0.51	7.94	7.86	789	588
5	Sarna	" "	" "	6.48	540.06	0.92	6.20	7.5	809	136
6	Rohnakala	" "	" "	5.87	739.29	0.73	8.96	9.6	348	322
7	Bohna	" "	" "	4.37	447.0	0.94	1.7	7.2	745	786
8	Bangoan	" "	" "	9.0	264.35	0.56	11.96	7.66	798	284
9	Linga	" "	" "	2.40	802.26	0.76	6.83	7.6	643	431
10	Silwani	" "	" "	0.39	474.38	0.82	3.37	7.35	1722	596

Source- P.H.E. Department Chhindwara.

Table 4: Ground water analysis of different villages of Pandhurna block

Sr. No.	Name of village	Volume	Source	Fluoride 1.00/1.50	Salinity 200/1000	Iron 0.10/1.00	Turbidity 2.50/10.00	pH 6.50/8.60	EC	Hardness 200/600
1	Sawajpani	100ml each	Hand pump	13.70	778.82	0.39	10.03	7.9	709	744
2	Mundidana	"	" "	11.48	378.69	0.85	20.41	7.84	805	362
3	Salai	" '	"	9.10	221.48	0.47	11.02	7.8	707	396
4	Siratha	" '	" '	10.0	357.74	0.56	8.24	7.86	979	568
5	Badchicholi	" "	" "	5.0	550.06	0.90	10.20	10.5	1009	536
6	Bangaon	" "	" "	5.5	639.29	0.78	8.96	9.6	349	389
7	Gujerkhedi	" "	" "	4.70	347.0	0.99	11.7	11.2	845	686
8	Pither Raiyt	" "	" "	10.4	1164.35	0.41	13.96	7.8	759	387
9	Pandhurna	" "	" "	0.36	882.26	0.50	26.83	7.83	446	536
10	Biroli	" "	" "	4.50	414.38	0.79	13.37	7.6	667	396

Source- P.H.E. Department Chhindwara.

Table 5: Ground water analysis of different villages of Churai block

Sr. No.	Name of village	Volume	Source	Fluoride 1.00/1.50	Salinity 200/1000	Iron 0.10/1.00	Turbidity 2.50/10.00	pH 6.50/8.60	EC	Hardness 200/600
1	Sitapar	100ml each	Hand pump	2.24	768.82	0.76	7.03	7.93	332	644
2	Dawazir	"	" "	5.45	388.69	0.85	2.41	7.84	1005	362
3	Bichhwa	"	"	11.40	281.48	0.49	9.02	7.7	779	476
4	Sihoramal	"	"	12.20	257.74	0.55	7.24	7.6	679	508
5	Moari	" "	" "	9.0	450.06	0.92	8.20	8.5	909	736
6	Kewlari	" "	" "	15.70	669.29	0.73	8.96	9.9	341	329
7	Hiwarkhedi	" "	" "	15.75	337.0	0.94	10.72	7.4	885	746
8	Pindrai saraf	" "	" "	15.07	764.35	0.46	3.96	7.64	726	384
9	Maduadhana	" "	" "	16.05	892.26	0.56	6.93	8.5	365	556
10	Khatkar	" "	" "	10.4	614.38	0.72	3.38	7.8	567	390

Source- P.H.E. Department Chhindwara.

One of the main trace elements in groundwater is fluoride which generally occurs as a natural constituent. Bedrock containing fluoride minerals is generally responsible for high concentration of this ion in groundwater. Fluoride normally accumulates in the bones, teeth and other calcified tissues of the human body. Excess of fluoride in water causes serious damage to the teeth and bones of the human body, which shows the symptoms of disintegration and decay, diseases called dental fluorosis, muscular

fluorosis and skeletal fluorosis. Higher intake of fluoride may change the metabolic activities of soft tissues (brain, liver, kidney, thyroid and reproductive organs). The permissible limit of fluoride in drinking water is 1.5 mg/l as per BIS standards. According to UNESCO specifications, water containing more than 1.5 mg/l of fluoride cause mottled tooth enamel in children and are not suitable for drinking purpose.

REFERENCES

The Public Health Engineering Department, Chhindwara.

Bhaskara Rao G. (2000) Ground water management studies, in Mohkhed, Chhindwara & Chourai blocks of Chhindwara district by the Central Ground Water Board during Annual Action Plan 1999-2000.

Bhaskara Rao G (2001) Ground water management studies, in Amarwara and Harrai blocks of Chhindwara district during Annual Action Plan 2000-2001.

Bhaskara Rao G (2002) Ground water management studies, in Tamia, Parasia, and Jamai blocks of Chhindwara district during Annual Action Plan 2001-2002.

Bhaskara Rao G (2003) Ground water management studies, in Bichhua, Sausar and

Pandhurna blocks of Chhindwara district during Annual Action Plan 2002-2003.

Kalra N, Kumar R, Yadav SS, Singh RT (2012) Physico-chemical analysis of groundwater taken from five blocks (Udwantnagar, Tarari, Charpokhar, Piro, Sahar) of southern Bhojpur (Bihar), *Journal of Chemical and Pharmaceutical Research*, 4(3):1827-1832.

Juneja T, Chaudhary A (2013) Assessment of water quality and its effects on the health of residents of Jhunjhnu district, Rajasthan: A cross sectional study. *Journal of Public Health and Epidemiology,* 5 (4): 186-191.

District Ground Water Booklet, (2007).

BIS, (1994) Indian Standards Specifications for Drinking Water, Bureau of Indian Standards, IS: 10500 -1994).

WHO., (1996) Guidelines for drinking-water quality, health criteria and other supporting information, V.2, 2nd edn, World Health Organization, Geneva, pp 940-949

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Studies on effects of dietary aflatoxin on growth performance and survival rate of fish *Clarias batrachus*

Fatmi Amjad[1*] and Ruby Durreshahwar[2]

[1]Department of Zoology , Govt. College, Dholpur-328001. Rajasthan, India
[2]Department of Zoology B. S. College Danapur, Patna -800014, Bihar, India
*Corresponding author Email :amjadfatmi@gmail.com

Manuscript details:

Received: 22.02.2016
Revised 18.03.2016
Accepted: 22.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Fatmi Amjad and Ruby Durreshahwar (2016) Studies on effects of dietary aflatoxin on growth performance and survival rate of fish *Clariasbatrachus*, *International J. of Life Sciences*, 4(1): 121-124.

Acknowledgement:
I am very much thankful to the Principal, Vice Principal and colleagues of department of Zoology and department of Botany for their cooperation in completion of this work. Thanks are also due to staff members of ICAR New Delhi, university of Rajasthan, AMU, Doctors and Technicians of veterinary Hospitals at Dholpur and Patna for their supports.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

Studies were conducted to determinate the effect of different levels of aflatoxin contaminated feed on growth performance and survival rate of fish *Clarias batrachus*. There was a significant decrease in average length gain, total weight gain, average weight gain as well as survival rate with increase in contamination of aflatoxin in the feed of the fish.

Keywords: Aflatoxin, Survival Rate, Average length gain, Average weight gain, Specific growth rate.

INTRODUCTION

Aflatoxins are metabolic by products of moulds *Aspergillusflavus* and *Aspergillusparasiticus.*These are toxic compounds and the cause of high mortality in animals and in some cases human beings (Reed and Casali, 1987, Montesano et al, 1995).The moulds can grow on the improperly stored feed or on feeds of inferior quality resulting in production of aflatoxins in many parts of the world including India. These toxins are completely heat stable so neither heating nor freezing destroy them and thus remain on the food indefinitely. The moulds grow on grains and legumes mostly during storage which then enters into human directly through food of plant origin and indirectly through food of animal origin. Toxigenic *A. flavus* produces aflatoxin B_1 and aflatoxin B_2 whereas *A. parasiticus* produces aflatoxin G_1 and aflatoxinG_2. AflatoxinB_1(AFB_1) is the most potent among all the types of aflatoxin identified till now and is classified as group 1 carcinogen by International agency for research on cancer. Effects of aflatoxin B_1 in fishes have been reported by several workers. Nunez *et al.*(1991) reported hepatocellular adenoma and hepatocellular carcinoma in Rainbow trout when exposed to aflatoxin B_1. Cagua*net al.*(2004) reported loss of appetite, decreased mean total biomass and low survival percentage in tilapia when fed with aflatoxin contaminated feed . There are reports of elevated liver function enzymes SGPT and SGOT in *Tilapia zilli* and *Clariaslazera* when these fishes were exposed to aflatoxinB_1 (Zaki*etal*., 2010; 2011). Mahfouz and Sharif (2015)

reported decrease growth indices and elevated ALT, AST and alkaline phosphatise in *Oreochromisniloticus* when exposed to various doses of AFB_1.

Asian cat fish *Clariasbatrachus* commonly known as Magur is an important commercial fish for its high quality flesh and for its medicinal value. In many parts of India it is consumed by anaemic, malnourished individuals, elderly persons and children due to its high nutrition value. Growth and survival are indicators of effects of a toxic compound in various animals including fish .The objective of the present study was to explore the effects of aflatoxin contaminated feeds on growth and survival of *Clariasbatrachus*.

MATERIALS AND METHODS

A total 72 apparently healthy *Clariasbatrachus* were obtained from private fish farm at Dholpur district of Rajasthan. The length of the fish was about 10 to 20 cm and the weight was about 35 to 55 grams. The fishes were kept in twelve aquaria measuring 2^1 X 1^1 X 1^1 . Six fishes were kept in each aquarium. Three aquaria were kept as control and nine aquaria were divided into three sets of three aquaria each and kept as experimental sets.

Preparation of Feed

Four types of feeds were prepared for the fishes depend upon the percentage of contaminated feed and they were distinguished as Feed I, Feed II, Feed III and Feed IV.

Feed I or good feed contained 100 percent good feed and no mouldy feed. Feed I were given to control or fishes of first set of aquaria comprising IA, IB and IC.

Feed II consisted of 90 percent good feed and 10 percent mouldy feed. Feed II were given to second set of aquaria comprising 2A, 2B and 2C.

Feed III contained 50 percent mouldy feed and 50 percent good feed. Feed III were given to fishes of third set of aquaria comprising of 3A, 3B and 3C.

Feed IV contained 100 percent mouldy feed. Feed IV was given to fishes of fourth set of aquaria comprising 4A, 4B and 4C.

Mouldy feed was prepared in laboratory. The commercial fish feed procured from market was first sprinkled with small amount of tap water to make the feed moist and then mixed with cultured *Aspergillusflavus* procured from ICAR New Delhi. The inoculation was made in a transfer chamber to avoid contamination. The mixed feed was then covered with a plastic sac. The infected feed was kept in a condition which is favourable for the growth of mould. Required amount of mouldy feed and good feed were weighed carefully for each treatment and then mixed thoroughly. The feeding was started from the second day two times a day at a feeding rate of 4% of the body weight. Growth rate were determined by using the method of Castle and Tiews (1980).

RESULTS AND DISCUSSION

Body Weight and body length

The result showed significant effects of aflatoxin on growth indices of the fish.

Body length gain was significantly ($p>0.05$) decreased in the fish fed with aflatoxin contaminated feed as compared to control. The total length gain in fishes fed

Table -1 Showingthe effects of aflatoxincontaminated feed on body weight , body length and survival rate in various groups of the fishes.

Treatment	I	II	III	IV
Initial Body length (cm)	11.36 ±1.15	11.67 ±2.21	10.95 ±1.12	11.60 ±1.12
Final body length	21.6 ±2.06	21.7 ±2.05	17.1 ±0.92	15.4 ±0.81
Initial body weight (g)	50.9 ±2.91	50.7 ±2.33	51.4 ±2.13	52.8 ±1.86
Final body weight (g)	125.7 ±3.34	124.0 ±3.37	109.3 ±2.31	98.7 ±3.26
Survival %	100	83	61	38

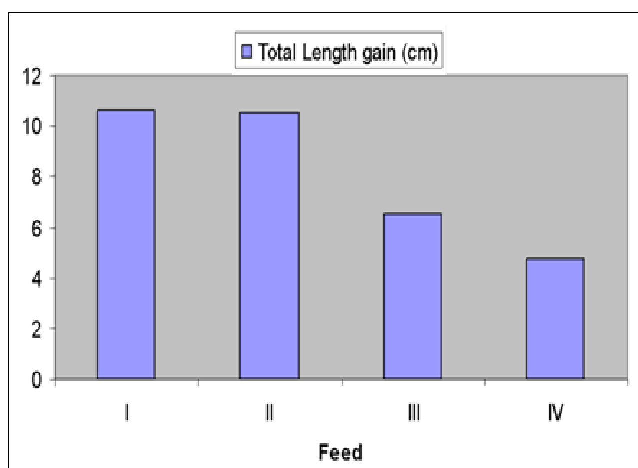


Fig. 1: Showing effects of aflatoxin on total length gain of the fish.

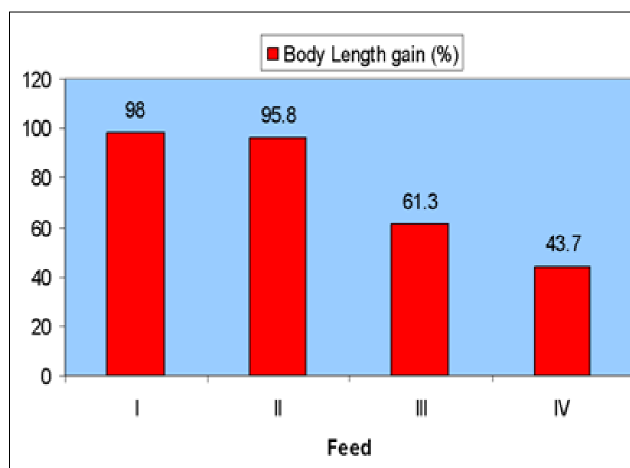


Fig. 2: Showing effect of aflatoxin on body length gain percent in the fish.

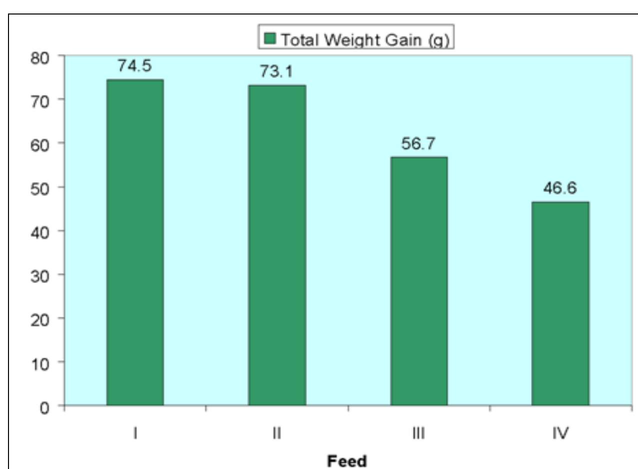


Fig.3:Shows effect of aflatoxin on total weight gain of the fish

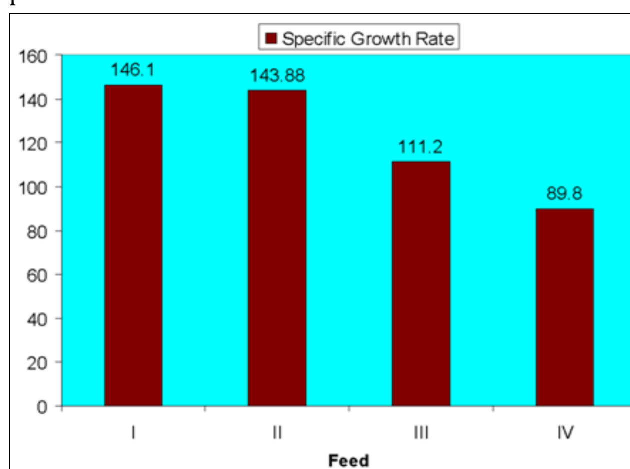


Fig. 4: Showing effect of aflatoxin on specific growth rate of the fish

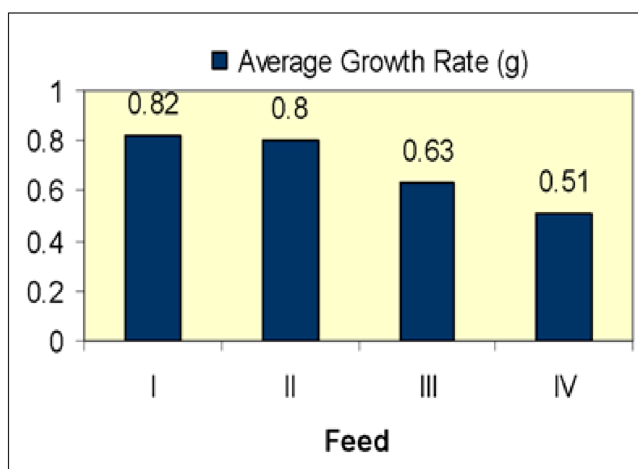


Fig. 5: Showing effect of aflatoxin on average growth rate of the fish

with feed I was 10.61 ±2.03 as compared to 4.7 ± 0.8 in fishes treated with feed IV. The percent length gain was also significantly higher in control or fishes fed with mould free feed as compared to fishes fed with feed IV hundred percent mouldy feed (fig 1 and 2).

The results of body weight gain are presented in table I and fig 3 - 5. The total weight gain (TWG) was 74. 5 ± 5.18 in fishes fed with feed I which gradually decreased with increase in the percentage of mouldy feed reaching its minimum 46.6 ± 3.75 in fishes fed with feed IV. The average growth rate (AGR) in fishes treated with feed I was significantly higher ($p>0.05$) than those of fishes which were fed with feed II, III and feed IV. The average growth rate in control was 0.82 ± 0.05 followed by 0.80 ± 0.03 in fishes treated with feed II and the minimum 0.51 ± 0.03 was found in fed with feed IV or hundred percent mouldy feed. The Specific growth rate (SGR) also showed a significant ($p> 0.05$) and gradual decline with the increase in the aflatoxin

contamination in the feed reaching its minimum in those fishes which were given feed IV or hundred percent mouldy feed. These results agree with the findings of Royes*et al.* (2002), Caguan*et al.* (2004), Mahfouz and Sharif (2015) in *O. niloticus*, Ogunjobi*et al*.(2012), Agbebi*et al*. (2012) in *C. gariepinus*.

Aflatoxin causes decreased appetite in in fishes (cheek and Shull, 1985) and also suppresses activities of different cell proteins which leads to inhibition of protein synthesis as well as lipid and carbohydrate metabolism (Joner 2000). Thus negative effects of aflatoxin on growth in the present studies may be due to suppression of metabolic process of carbohydrate, lipid and protein and also due to loss of appetite as a result of aflatoxin.

Aflatoxin may also create a condition of stress increasing utilization of glutathione for detoxification process in fishes (Devegowda, 1998). Methionine is one of the components glutathione. Hence this process of detoxification may decrease availability of methionine resulting in poor growth.

Survival Rate

Survival rate decreased with increase in aflatoxin contamination in feeds. The fishes which were fed with feed I or aflatoxin free diet showed maximum or 100% survival whereas minimum survival i.e., 38% was observed in those fishes which were given feed IV (Table 1). The present findings are in agreement with those found in *O. Niloticus*(Caguan*et al*., 2004; Mahfouz *et al*., 2015).

Aflatoxin causes hepatic abnormality in fishes (Nunez *et. al*., 1991; Zaki*et al*., 2011; 2012). Thus the lowered survival rate in aflatoxin treated fish was probably due to impaired liver function and loss of appetite.

Zaki*et al*. (2012) reported decreased immunity in *Clariaslazera* when exposed to aflatoxin. Thus the decreased in survival rate may also be due to loss of immunity in the aflatoxin treated fishes.

REFERENCES

Agbebi OT, Lawal HB and odebiyi OC (2012) Aflatoxin effect of moulded gala waste mixed with ginger and its histopathological study on *Clariasgariepinus*. *Glo. J. Inc. (US)*. 12(11), 8:16.

Caguan AG, Tayaban RH, Somga JR And Bartolome RM (2004) Effect of Aflatoxin Contaminated feed in Nile tilapia (*Oreochromisniloticus* L.). In *Proceeding of the 6th International symposium on tilapia in aquaculture (R.B. Remedios, G.C. Imir and K. Fitzsimons.ed)*, **pp**. 172–178

Castle JD and Tiews K (1980) Reports of the EIFAC, IUNS and ICES working group on standradization of methodology in fish nutrition research. Hamburg, Fedral Republic of Germany,EIFAC. Technology, 36 : 24.

Cheeke PK and Shull LR (1985) Natural Toxicant in feeds and poisonous plants. *Abi.Publishing company.ING.West Port. Connecticut.*

Devegowda G, Raju MLNV, Afzali N and Swamy HVNL (1998) Mycotoxins picture worldwide .Novel solution for their counter action. In T. P. Lyons and K. A. Jacques (Eds) Biotechnology in the feed industries.Pp 241-255.Proc.Of All tech's 14.The annual symposium.Nottingham. U.K.

Joner A (2000) Mycotoxine*(http : // www. Ansci Cornell. Edu / courses / as625 / 1999 term / toner / aflatoxin. Html)*

Mahfouz ME and Sharif AH (2015) A multiparameter investigation into adverse effects of aflatoxin on *Oreochromisniloticus* health status.*J. Bas. Appl. Zool*.,71: 48-59.

Montessano R, Hainut P and Wild CP (1997) Hepatocellular carcinoma: From gene to public health review. J. Nat. Cancer Inst. 89: 1844-1851.

Nunez JDH, Hendricks JD and Duimishra JR (1991) Ultra structure of hepatocellular neoplasms in aflatoxin B_1 (AFB_1) initiated rainbow trout (*Oncorhychusmykiss*). *ToxicolPathol,*.**19**:11-21

Ogungabi AA, Owoseni MC, Bello OS, Ewvola EO, Adeleke AJ (2012) Growth performance and survival rate of *Clariasgariepinus*juvenile fed different levels of aflatoxin contaminated feed. *Afri. J. O. L*.6 (4)

Reed JD and Casali OB (1987) Hazards to livestock consuming aflatoxin contaminated meal in Africa. In: *ICRISAT proceeding of international workshop on aflatoxin contamination in ground nut.*6-9 Oct.1987.

Royes JB and Yanong RPE (2002) Molds in fish and aflatoxin*(http \ edie. 1 fas. Vtrl.edu. \ FA095*).

Zaki MS, Fawzi OM, Omer S, Khafagy M, Fawzy M and Awed I (2010) Diminution of aflatoxicosis in *Tilapia zilli*fish by dietary supplementation with fix in toxin and Nigella sativa oil. *Nat. Sci*., 8(2) : 43- 49.

Zaki MS, Fawzy OF and Imam MZ (2011) Reduction of aflatoxin in *Clariuslazera* cat fish by Ginseng extract and *Nigella sativa* oil. *J American Sci*., **7**: 591-596.

Zaki MS and Fawzy OF (2012) Effect of afla- Toxins B_1 on endocrine status in cat fish(*Clariouslazera*). *Life.Sci.J.*; 9(1) :419-422.

.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Zooplankton Diversity of a freshwater Pond in a Cachar district of Assam, India

Kar S[1]* and Kar D[2]

[1]Research Scholar, [2]Professor, Department of Life Science and Bio-informatics, Assam University, Cachar-788007, Assam.

*Corresponding Author: kar_sulata@rediffmail.com

Manuscript details:

Received: 25.12.2015
Revised 30. 01.2016
Accepted: 09.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Kar S and Kar D (2016) Zooplankton Diversity of a freshwater Pond in a Cachar district of Assam, India, *International J. of Life Sciences*, 4(1): 125-128.

Acknowledgement:
First author is very much thankful to the Department of Life Science and Bio-informatics, Assam University, Silchar for providing the laboratory for working and Department of Zoology, Ballygunge Science College, Calcutta University for the help and assistance during the study. First author is also thankful to Assam University for providing her the scholarship as a Research Scholar.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

Narsingtola pond is located at Silchar, Cachar district of Assam having rich diversity of aquatic vegetation though situated amidst the highly polluted area of the town. The present study deals with the Zooplankton diversity of the Narsingtola pond in Cachar district of Assam. The survey was carried out four times in a month during morning hours from September 2014 to August 2015. During the study, 42 genera of Zooplankton were recorded from the site of which 13 genera belongs to Cladocera, 4 genera belongs to Copepoda and 25 genera belongs to Rotifera. Abundance status and population density of the Zooplankton groups were also recorded. Rotifera was the dominant group among Zooplankton community constituting 42% of the Zooplankton population. Higher population density of Zooplankton were recorded during winter season and lower during the summer season.

Keywords: Diversity, Narsingtola pond, Zooplankton, Rotifera.

INTRODUCTION

Zooplankton are a group of important organism of key importance in regulating patterns and mechanisms through which matter, energy and pollutants are transferred from the base to the upper levels of an aquatic food webs. It plays a crucial role in aquatic ecosystem as they are cosmopolitan in nature and they inhabit all freshwater habitats of the world (Gadekar, 2014). Ecologically Zooplankton are one of the most important biotic components influencing all the functional aspects of an aquatic ecosystem such as food chains, food webs, energy flow and cycling of matter (Dadhick and Sexena, 1999; Park and Shin, 2007). Their flucations in occurrence and abundance can be used to estimate the fishery potential of a water body (Hutchinson, 1967). Zooplankton includes most favourite prey of fish which, at least during early life stages, substantially feed on them. Predation by fish and vertebrates is, in general, visual, therefore depending on prey size and visibility (de Bernardi *et al.*, 1987). Thus, for better understanding of life in any aquatic body, knowledge of Zooplankton diversity is a must requirement.

Various works on Zooplankton are being reported from throughout India and also on freshwater bodies of different parts of Northeast India (Sharma and Sharma, 2008)but in Cachar, Assam there is very scarcity of report on Zooplankton diversity except some worthmentioning of Kar and Barbhuiya (2004) and Kar (2007). So, the present study was an attempt for investigating Zooplankton diversity of Narsintola pond, Cachar, Assam.

MATERIAL AND METHODS

Study area: Narsingtola pond is situated at Silchar town of Cachar district, Assam. It lies between 24°49´36.6˝N and 92°47´58˝E. It has large human interference as situated at highly polluted market area of Silchar town, surrounded by various kinds of shops and vendors.

Zooplankton sampling: The study was conducted for a period of one year from September 2014 to August 2015. The sampling protocol included weekly sampling of Zooplankton from the site following standard literature of Battish (1992). After collection, Zooplankton were filtered, placed in Tarson (100 ml) container and subsequently fixed in Lugol's solution and stored in cool and dark place. For studying the diversity of Zooplankton, sample were taken in a Sedgwick-Rafter counting chamber and observed under a light microscope under required magnification (X 10 intially , followed X 40) and the specimens were identified following Battish (1992); Edmondson (1959); Michael and Sharma (1998); Sharma (1998); Sharma and Sharma (2008).

RESULTS AND DISCUSSION

During the present investigation, 42 genera of Zooplankton were recorded from the site belonging to three major groups. Among the recorded species, 13 genera belongs to Cladocera, 4 genera belongs to Copepoda and 25 genera belongs to Rotifera (Table 1).

Table 1: Zooplankton species abundance of Narsingtola pond, Cachar, Assam

Zooplankton	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
CLADOCERA												
Diaphanosoma sp.	+	+	+	+	+	+	+	+	+	+	+	+
Scapholeberis sp.	+	+	-	-	+	-	-	+	+	+	+	+
Sida sp.	+	+	+	+	+	+	+	+	+	+	+	+
Simocephalus sp.	+	+	+	+	+	+	+	+	+	+	+	+
Macrothrix sp.	-	+	-	+	+	-	-	+	-	+	+	+
Chydorus sp.	+	+	+	+	+	+	+	+	+	+	+	+
Ceriodaphnia sp.	+	+	+	+	+	+	+	+	+	+	+	+
Bosmina sp.	+	+	+	+	+	+	+	+	+	+	+	+
Bosminopsis sp.	-	+	-	-	+	+	-	+	-	-	+	+
Alona sp.	+	+	+	+	+	+	+	+	+	+	+	+
Alonella sp.	+	-	+	+	-	-	-	-	-	+	+	+
Daphnia sp.	-	+	-	-	-	-	-	-	-	-	-	+
Moina sp.	+	+	+	+	+	+	+	+	+	+	+	+
COPEPODA												
Mesocyclops sp.	+	+	+	+	+	+	+	+	+	+	+	+
Thermocyclops sp.	+	+	+	+	+	+	+	+	+	+	+	+
Neodiaptomus sp.	+	+	+	+	+	+	+	+	+	+	+	+
Heliodiaptomus sp.	+	+	+	+	+	+	+	+	+	+	+	+
ROTIFERA												
Brachionus sp.	+	+	+	+	+	+	+	+	+	+	+	+
Philodina sp.	-	-	-	+	-	-	-	-	-	-	+	-
Plationus sp.	+	+	+	-	+	-	+	-	-	+	-	+

Table 1: Continued…

Zooplankton	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
ROTIFERA												
Lecane sp.	+	+	+	+	+	+	+	+	+	+	+	+
Lepadella sp.	-	+	-	+	-	+	+	+	+	-	+	+
Keratella sp.	+	+	+	+	+	+	+	+	+	+	+	+
Anuraeopsis sp.	-	+	+	+	+	+	+	+	+	+	+	+
Asplanchna sp.	+	+	+	+	+	+	+	+	-	+	+	+
Ascomorpha sp.	+	+	+	+	+	+	+	+	+	+	+	+
Testudinella sp.	+	+	+	+	+	+	+	+	+	+	+	+
Trichocerca sp.	+	+	+	+	+	+	+	+	+	+	+	+
Trichotria sp.	-	-	-	-	-	-	-	-	-	-	+	-
Taphrocampa sp.	-	-	-	+	-	-	-	-	-	-	-	-
Cephalodella sp.	+	+	+	+	+	+	+	+	+	-	+	+
Macrochaetus sp.	-	-	-	-	-	-	-	-	-	-	-	+
Mytilina sp.	-	-	-	-	+	-	-	-	+	-	+	+
Horaella sp.	-	-	-	-	+	-	-	+	-	-	-	+
Filinia sp.	+	+	-	+	+	+	-	+	+	+	+	+
Colurella sp.	-	+	-	+	-	+	-	+	-	+	-	+
Trochosphaera sp.	-	+	-	-	-	-	-	-	-	-	+	-
Conochilus sp.	-	-	-	+	-	-	-	-	-	-	-	-
Rotaria sp.	-	-	-	+	-	-	-	-	+	-	-	+
Scaridium sp.	+	+	+	+	+	+	+	+	+	+	+	-
Platyias sp.	-	-	-	+	-	-	-	-	-	+	-	-
Synchaeta sp.	-	-	-	-	-	-	+	-	-	-	-	+

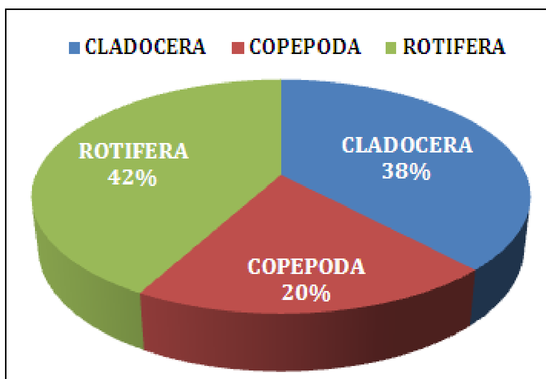


Fig 1: Abundance status of Zooplankton species of Narsingtola pond, Cachar, Assam

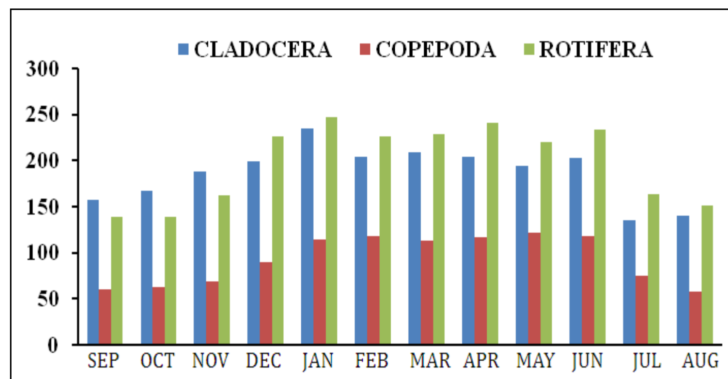


Fig 2: Groupwise population density status of Zooplankton of Narsingtola pond, Cachar, Assam

The abundance status of Zooplankton groups were also recorded (Fig 1). During the present study, Cladocera constituted 38% of the total identified Zooplankton of which *Diaphanosoma sp., Sida* sp., *Simocephalus* sp., *Chydorus* sp., *Ceriodaphnia* sp., *Bosmina* sp., *Alona* sp. and *Moina* sp. were recorded in all seasons.Copepoda constituted 20% of the total identified zooplankton of which recorded four groups from the site viz, *Mesocyclops* sp., *Thermocyclops* sp., *Neodiaptomus* sp. and *Heliodiaptomus* sp. were recorded in all seasons. Rotifera constituted 42% of the total identified Zooplankton of which *Brachionus* sp., *Lecane* sp., *Keratella* sp., *Ascomorpha* sp., *Testudinella* sp. and *Trichocerca* sp. were recorded in all seasons. In the present study Rotifera group of Zooplankton community was found dominant with 25 genera constituting 42% of the total Zooplankton population. Similar observation was made by Tyor *et al.* (2014) during their study of Zooplankton diversity in a shallow lake of Gurgaon, Haryana. Rotifera was

followed by Cladocera and then Copepoda showing least diversity and dominance with only 4 genera constituting 20% of the total Zooplankton population.

Population density status of the recorded Zooplankton were depicted in Fig 2. Higher population density of Zooplankton were recorded during winter season and low during the summer season. Density of Cladocera was found to be higher in the month of January and lowest in the month of July, similarly Rotifera showing peak at the month of January and lowest at the month of September and October. Density of Copepoda were recorded to almost similar throughout the study period except slightly lower in summer season and slightly higher in winter season.

Many researchers from South Assam such as Kar and Kar (2013) reported 26 species of Zooplankton from an oxbow lake of Cachar, Narzary *et al.*(2015) reported 20 genera of Zooplankton from three different wetlands of Cachar district, Sonowal *et al.*(2015) reported 19 species of Zooplankton from three sites of Southern Assam, Suganthi *et al.*(2014) on a study on Zooplankton in 5 freshwater ponds of Cuddalore district, Tamil Nadu, reported 25 species of Zooplankton, a total number of 78 Zooplankton species were reported from a reservoir of West Bengal by Bera (2014), Pawar (2014) reported 66 species of Zooplankton in some freshwater bodies around Satara district of Maharashtra, India.

CONCLUSION

Zooplankton diversity of the Narsingtola pond revealed during the present study confirm the habitat to be rich for Zooplankton population and suitable for aquaculture, as Zooplankton are known to be the best food for fish larvae in aquaculture. Although the aquatic habitat of the Pond is being highly disturbed by different anthropogenic disturbances like washing clothes and utensils, direct bathing in the pond, dumping of different kinds of waste materials from the shops and vendors nearby the Pond etc., yet there is diverse presence of the Zooplankton in Narsingtola pond. Thus, keeping the above in view, steps should be taken for maintenance and conservation of Narsingtola pond.

REFERENCES

Tiple Ashish D and Khurad Arun M (2009) Butterfly species diversity, Habitats and seasonal distribution in and around Nagpur City, Central India. World Journal Zoology 4 (3): 153-162.

Tiple Ashish D (2011) Butterflies of Vidarbha region, Maharashtra State, central India. Journal of Threatened *Taxa* 3(1): 1469-1477

Battist A (1988) Phytophagous insects in the energy flow of an artificial stand of Pinus nigra Arnold in North Italy. Redia, 71(1): 139-160.

Chakaravarthy AKD (1997) Rajagopal and R. Jagannatha: Insects as bio indicators of conservation in the tropics. *Zoo's Print J.*, 12, 21-25.

Gunathilagaraj K, Perumal TNA, Jayaram K and Kumar MG (1998) Some South Indian Butterflies. Nilgiri Wildlife and Environment Association, Tamil Nadu, India. 274pp.

Jana S, Pahari PR, Dutta TK and Bhattacharya T (2009) Diversity and community structure of aquatic insects in a pond in Midnapore town, West Bengal, India. *J. Environ. Biol.*, 30, 283-287.

Krebs C (1989) Ecological Methodology. HarperCollins, New York, 654pp.

Kunte K (2009) Occurrence of *Elymnias obnubila* Marshall and de Nicéville, 1883 (Lepidoptera: Nymphalidae: Satyrinae) in southern Mizoram: Range extension of the species and an addition to the Indian butterfly fauna. Journal of Threatened *Taxa* 1(11): 567-568.

Kunte K (2000) Butterflies of Peninsular India. Indian Academy of Sciences, Universities Press (India) Limited. 254.

Magurran AF (1988) Ecological Diversity and Its Measurements. University Press, Cambridge, 192pp.

May PG (1992) Flower selection and the dynamics of lipid reserves in two nectarivorous butterflies. Ecology, 73: 2181-2191.

Odum EP (1997) Ecology: A Bridge between Science and Society. Sinauer Associated Inc. Sunderland, Massachusetts, USA, 330pp

Palot Muhamed Jafer and Soniya VP (2003) A Preliminary Report on the Butterflies of Lonar Crater Lake, Buldhana District, Maharashtra. *Zoos' Print Journal*, 18 (11): 1267-1268

Pielou EC (1969) An Introduction to Mathematical Ecology. John Wiley, New York, 286pp.

Raup DM (1994) The role of extinction in evolution. Proceedings of the National Academy of Sciences 91 (15): 6758–6763.

Sala Osvaldo E, Meyerson Laura A and Parmesan Camille (2009) Biodiversity change and human health: from ecosystem services to spread of disease. Island Press. 3–5.

Shannon CE (1948) A mathematical theory of communication.Bell System Technical Journal 27: 379-423 & 623-656.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

The ecological impact of invasive Trematode Parasites of Common toad (*Bufo melanostictus)* found in bank of Saryu River along Chapra town and effect of flanking population

Seema Kumari, Vishwranjan and Prashant Kumar

P.G. Department of Zoology, J.P. University Chapra, India
Email: cprseema@gmail.com, vishwranjan@yahoo.com, prashant_kumar147@rediffmail.com

Manuscript details:

Received: 08.02.2016
Accepted: 08.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Seema Kumari, Vishwranjan and Prashant Kumar (2016) The ecological impact of invasive Trematode Parasites of Common toad (*Bufo melanostictus)* found in bank of Saryu River along Chapra town and effect of flanking population, *International J. of Life Sciences*, 4(1): 129-132.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

Incidence of Trematode Parasites of Common tode (*Bufo melanostictus*) has been studied in bank of Saryu River along Chapra town during 2010-2013. Chapra is the District Head quarter of Saran. This study shows the Trematodes found in common tode of this area and this is worse condition of the population situated near the bank of Saryu River. Trematodes can be found anywhere where untreated water and human waste is spread out. A simple food chain and contamination of food and water is follows the infectious route. Out of total 1328 Toades (*Bufo melanostictus*) collected and examined, trematodes were 450 (57.3 per cent). The rate of infection of trematodes was higher in sex-wise female's 256 (34.5 per cent) males 194 (33.1 per cent). Pollution due to sewage and excreta waste of town increase the infection of toad and flanking population.

Keywords: *Bufo melanostictus*, Trematode, Saryu River, Bufotoxins

INTRODUCTION

Ecological Parasitology according to Dogiel (1964), deal with the study of relationship between the parasitic fauna of the host taken as a unit and the changes in the environmental and physiological conditions of the host. However, much work has been done on the general incidence of helminth parasites of vertebrates, taken as groups, viz., exploring the helminth fauna of fishes, amphibians, reptiles, birds and mammals, together with their ecological variations. The helminthologists of the country in general and the state of Bihar in particular have not yet reported the parasites fauna of the host and its impact on human population. Though some studies in this direction have been undertaken in recent past by various workers. It is, therefore intended to carry on this type of work, viz., ecological parasitology of selected vertebrate host, viz., Toad (*Bufo melanostictus*), commonly available in bank of Saryu River along Chapra town and effect of flanking population.

MATERIALS AND METHODS

Toads (*Bufo melanostictus*) were collected from near the houses, drainages, River bank of Saryu , playing boys in and around Chapra town,Bihar during the period of three year May 2010 to May 2013.

The toads were immediately transferred to the laboratory, sacrificed and dissected as soon as possible. All viscera were removed and each placed in petri dish with normal physiological saline. The viscera were examined for helminths under a dissecting microscope. The parasites recovered from each toad were collected and counted. Details like; location, number, species of the parasites and month of collection. The lungs were cut and checked out for infection. The recovered helminths were cleaned carefully. Trematodes were fixed under pressure of cover glass in AFA solution. Cestode parasites were fixed in AFA in between two slides or glass plates tied together at the ends by rubber bands and placed two hours in a jar or petri dishes containing the fixative. Specimens of trematodes and cestodes were then transferred to separate vials containing the fixative. After 24 hours the fixed worms were preserved in 70% alcohol in separate vials. For anatomical study, both Trematodes and Cestodes were stained in Semichon's Solution. The nematodes were fixed in 70% alcohol and cleared in lactophenol.

RESULTS AND DISCUSSION

Out of total 1328 Toads (*Bufo melanostictus*) collected and examined, trematodes were 450 (57.3 per cent). Sex-wise, the rate of infection of trematodes was higher in females 256 (34.5 per cent), than the males 194 (33.1 per cent).

Table 1: The Sex-wise, the rate of infection of trematodes

Sex	Tota;			No. of toads infected with		
				Trematodes	Percentage	
	Examined	Infected	%		Examined	Infected
Male	586	321	54.7	194	33.1	60.4
Female	742	463	62.3	256	34.5	55.2
Total	1328	784	59	450	33.8	57.3

Table 2: Month-wise, the rate of infection of trematodes

Month	Infection in			Male infected with Trematodes		Female infected with Trematodes		Total infected with Trematodes	
	Male	**Female**	**Total**	**No.**	**%**	**No.**	**%**	**No.**	**%**
May	21	27	48	17	80.9	24	88.8	41	85.4
June	46	47	93	24	52.1	29	61.7	53	56.9
July	53	54	107	28	52.8	34	62.9	62	57.9
August	51	66	117	31	60.7	37	56	68	58.1
September	49	79	128	26	53	38	48.1	64	50
October	48	98	146	21	43.7	27	27.5	48	32.8
November	20	25	45	19	95	20	80	39	86.6
December	-	2	2	-	-	-	-	-	-
January	-	-	-	-	-	-	-	-	-
February	7	20	27	7	**100**	10	50	17	62.9
March	11	17	28	9	81.8	16	94.1	25	89.2
April	15	28	43	12	80	21	75	33	76.7
Total	321	463	784	194	60.4	256	55.2	450	57.3

Table 3: Season-wise,the rate of infection of trematodes

Seasons	Examined	Infected	Percentage (%) (taken from examined hosts)	Both(Male & Female) Trematode	
				No.	%
Winter	194	74	38.1	56	12.4
Summer	431	212	49.1	152	33.7
Rainy	703	498	70.8	242	53.7
Total	1328	784	59	450	57.3

Table 4: Location-wise infection of Trematode Parasites in *Bufo melanostictus* (Toad)

Month	Infection in			Location																	
	Male	Female	Total	Rectum						Intestine						Lungs					
				M	%	F	%	Total	%	M	%	F	%	Total	%	M	%	F	%	Total	%
May	17	24	41	10	58.8	-	-	10	24.3	7	41.1	20	83.3	27	65.8	-	-	4	16.6	4	9.7
June	24	29	53	-	-	-	-	-	-	11	45.8	17	58.6	28	52.8	13	54.1	12	41.3	25	47.1
July	28	34	62	-	-	28	82.3	28	45.1	18	64.2	6	17.6	24	38.7	10	35.7	-	-	10	16.1
August	31	37	68	6	19.3	-	-	6	8.8	25	80.6	22	59.4	47	69.1	-	-	15	40.5	15	22
September	26	38	64	-	x	25	65.7	25	39	18	69.2	13	34.1	31	48.4	8	30.7	-	-	8	12.5
October	21	27	48	21	100	27	100	48	100	-	-	-	x	-	-	-	x	-	-	-	-
November	19	20	39	-	-	8	40	8	20.5	17	89.4	12	60	29	74.3	2	10.5	-	-	2	5.12
December	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
January	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
February	7	10	17	5	71.4	10	100	15	88.2	2	28.5	-	-	2	20	-	-	-	-	-	-
March	9	16	25	7	77.7	10	62.5	17	68	2	22.2	6	37.5	8	50	-	-	-	-	-	-
April	12	21	33	-	-	15	71.4	15	45.4	4	33.3	6	28.5	10	47.6	8	66.6	-	-	8	24.2
TOTAL	194	256	450	49	25.2	123	48	172	38.2	104	53.6	102	39.8	206	45.7	41	21.1	31	12.1	72	16

In the present study, females showed insignificantly higher (34.5 per cent) rate of infection than the male toads (33.1 per cent). The present finding is in conformity with the findings of Griffin (1989), while working on frog. Griffin (1989) has also mentioned about correlation between size of the host and the intensity of infection. The higher rate of infections in females could be due to their larger size; more feed requirement, hence prone to more parasites. This is also in agreement with the findings of Muzzall (1991).

However, the present finding is in contrary with the findings of Lees (1962), Begum and Banu (2012), while working on *Bufo melanostictus* of Dhaka city. Khurshid (2010), while working on carp, also reported the higher rate of infection in females than males.

Month-wise, the maximum (100 per cent) rate of infection was observed in male in the month of February and the minimum (43.7 per cent) in the month of October. In female, maximum (94.1 per cent) rate of infection was observed in the month of March and the minimum (27.5 per cent) was observed in the month of October.

Trematodes were found in minimum (89.2 per cent) number in the month of March. The present finding is in contrary with the finding of Rizvi and Bhutia (2009). These differences are statistically insignificant. As far as the rate of infection is considered and showed the minimum infection in the month of October the lowest (32.8 per cent) was in case of this helminth parasite.

Sex-wise, males toads were heavily (100 per cent) infected and in month of February. Similarly, in case of male, when lowest rate of infectionwas considered with respect to month of October (43.7 per cent). In case of females, (94.1 per cent) in the month of March and the minimum (27.5 per cent) rate of infection in the month of October.

Location-wise, the rate of infection of trematodes was maximum (45.7 per cent) from intestine, followed by the rectum (38.2 per cent) and the lungs (16 per cent). Location-wise the parasites were collected from intestine, rectum, stomach, lungs and gall bladder (no infection was observed from the buccal cavity, skin, liver and urinary bladder).

In the present study, the parasites were usually found along the digestive tract i.e. intestine, rectum and

stomach as these were observed as their favourite sites and believed to have a communalistic relationship with its host and did not pose any threat to the host even though they were found in abundance. These differences were found statistically significant.
Table- Location –wise:

Season-wise, the maximum (53.7 per cent) rate of infection was observed in rainy season followed by summer (33.7 per cent) while minimum (12.4 per cent) trematodes were found during the winter season. This observation is in conformity with the findings of Chandra and Gupta (2007); Begum and Banu (2012) The higher rate of infection in rainy season may be due to the damp habitats favoring the survival of infective stages of parasites.

During winter season, toads hibernate and there is a reduction in metabolism resulting in hypobiosis. The influence of hibernation can be more properly considered as being the sum of influence of several factors involving cessation of feeding. The drop in temperature retards reproduction and may lead to a reduction in the number of parasites.

The parasites of amphibians are still more affected due to seasonal and climatic factors because, firstly their hosts are cold-blooded and secondly, they are amphibious in nature, which directly affect the parasite population and the amphibians mode of life provides a wider exposure to both terrestrial and aquatic conditions, which favour parasites. These differences were found statistically insignificant.

However, the present finding is in contrary to the finding of Lees (1962), who reported the highest incidence, was in autumn.

Statistically, these differences were found insignificant. The reasons for the highest rate of infection in rainy season and lowest in winter season have already being mentioned, earlier in this chapter.

REFERENCES

Begum A and Banu N (2012) Sex organal and differences of Helminthofauna of toad, *Bufo melanostictus* (Schneider, 1799). *Bangladesh Journal of Zoology*, 40(2): 155-164.

Chandra P, Gupta N (2007) Habitat preference and seasonal fluctuations in the helminthofauna of amphibian hosts of Rohilkhand zone, India. *Asian J Exp Sci.*, 21:69–78.

Griffin CT (1989) *Oswaldocruziafiliformis*(Nematoda: Trichostrogyloidea) in frogs (*Ranatemporaria*) From three locations in Ireland. *Journal of Helminthology,* 63:53-62.

Hollis PD (1972) Host sex influence on the seasonal incidence of Haematolocehusmedioplexus (Trematoda: Plagiorchiidae) in *Ranapipiens. J. Parasitol*., 58:128.

Johnson PTJ and McKenzie VJ (2009) Effects of environmental change on helminth infections in amphibians: exploring the emergence of Ribeiroia and Echinostoma infections in North America. *The Biology of Echinostomes*, pp. 249-280.

Kadlec D, Simková A, Jarkosvk J and Gelnar M (2003) Parasitic communities of freshwater fish under flood conditions. *Parasitology Research* 89:22-283.

Kennedy CR and Lie SF (1974) The distribution and pathogenicity of stronglyllids (nematode) in brown trout salmotrutta (L) in Feraworthy Reservoir. *Devon. J. Fish Scot*. 8: 293-302.

Khurshid I, Chishti Z, Ahmad F, Tak H, Nazir B, Ahmad B and Rahid U (2010) Population and dispersal patterns of helminth parasites and their impact on hematology in fishes of Shall bug Wetland. *22nd National Congress of Parasitology*: 88-89.

Koller RL and Gaudin AJ (1977) Analysis of helminth infection in *Bufoboreas*(Amphibian: *Bufonidae*) and *Hylaregilla*(Amphibian: *Hylidae*) in Southern California. The Southwest Naturalist, 21 (4): 503-509.

Koprivnikar J, Baker RL and Forbes MR (2006) Environmental factors influencing trematode prevalence in Grey tree frog (*Hylaversicolor*) tadpoles in Southern Ontario. *Journal of Parasitology*, 92(5):997-1001.

Lees E (1962) The incidence of Helminth Parasites in a particular frog population. *Parasitology,* 52:95-10.

McAllister CT, Brusey CR and Freed PS (2010) Helminth parasites (Cestodea: Nematoda) of select Herpetofauna from Paraguay. *Journal of Parasitology* , 96 (1): 222-224.

Milinski M (1984) Parasites determine a predator's optimal feeding strategy; Behav. *Ecol.Sociobiol.*, 15, 35-37.

Moore J (1984) Altered behavioural responses in intermediate hosts- an Acanthocephalan parasite strategy. *Amer. Nat*., 123, 572-577.

Muzzall PM (1991) Helminthintracommunication of the Frogs *Ranacatesbeiana and Ranaclamitans*fromTurky marsh, Michigan. *J. Parasitol.*, 77 (3) p.366-371.

Muzzall PM, Gillill MG, Summer CS and Mehne CJ (2001) Helminth communities of green frogs *Ranaclamitanslatreille,* from Southwestern Michigan. *Journal of Parasitology*, 87(5):962-968.

Rizvi AN and Bhutia PT (2009) Helminth parasite communities in anuran amphibians of Kalesar Wildlife Sanctuary (Haryana), India. 20th All India Congress of Zool. Mumbai book of Abstracts: 191.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Changing biodiversity scenario in Lonar meteoritic crater, (MS), India, as revealed by the studies on insects (Order- Lepidoptera, Orthoptera and Odonata)

Pedge Sudarshan S and Ahirrao Sunil D

[1]Department of Zoology, Kai. Rasika Mahavidyalaya Deoni
[2]Department of Zoology, Shri Shivaji College Parbhani
*Corresponding author Email: pedge.sudarshan83@gmail.com

Manuscript details:

Received: 03.02.2016
Revised 22.03.2016
Accepted: 25.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Pedge Sudarshan S and Ahirrao Sunil D(2016) Changing biodiversity scenario in Lonar meteoritic crater, (MS), India, as revealed by the studies on insects (Order- Lepidoptera, Orthoptera and Odonata), *International J. of Life Sciences*, 4(1): 133-136.

Acknowledgement:
The authors are thankful to Principal and Head, Dept of Zoology. Shri Shivaji College, Parbhani- 431 401 (MS) India for provides laboratory facilities. We are grateful to UGC for providing financial assistance under JRF during the course of study. The authors are also thanks the Chief Wildlife Warden, Maharashtra Forest Department and his subordinates at Buldhana District for their cooperation

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

The Indian sub-regions hosts about 1,504 species of butterflies of which peninsular India hosts 351, and the Western Ghats 334. Lonar Lake (19°59'N & 74°34'E) is situated about 155km from Parbhani town in Buldhana District of Maharashtra State. The Lonar crater has a circular outline with a diameter of 1,830 m and a depth. This is the only one meteorite crater made by the basaltic rock in India. In the present investigation, 20 Arthropod species were found out of which 13 species of butterflies were reported and they belonging to 4 families of order Lepidoptera, 5 species of order Orthoptera and 2 species of order Odonata. Compare to previous studies of order Lepidopterans present conditions was drastically declining Lepidopteron diversity in the Lonar Lake.

Keywords: biodiversity, Lepidoptera Orthoptera, Odonata, Lonar Crater

INTRODUCTION

Biodiversity is the degree of variation of life forms within a given species, ecosystem, biome, or an entire planet. Biodiversity is a measure of the health of ecosystems. Biodiversity is in part a function of climate. In terrestrial habitats, tropical regions are typically rich whereas Polar Regions support fewer species. Rapid environmental changes typically cause mass extinctions. One estimate is that less than 1% of the species that have existed on Earth are extant (Raup, 1994). The period since the emergence of humans has displayed an ongoing biodiversity reduction and an accompanying loss of genetic diversity. The reduction caused primarily by human impacts, particularly habitat destruction. Conversely, biodiversity impacts on human health in a number of ways, both positively and negatively (Sala, 2009). In terrestrial ecosystem, insect fauna represent more than 70% and also play an important role in food chain for the natural balance. Insects are extremely important components of the bio-indicators of the world (Chakaravarthy *et al.*, 1997

Jana *et al*., 2009, Rajagopal *et al*., 2011). Insects comprise more than half of earth's diversity of species. Healthy biological communities totally depend on insects as pollinators, seed dispersers, herbivores, predators and prey. Within the ecological communities, insects comprise a large proportion of the biomass and are conditions of energy through the system (Battist, 1988; May, 1992; Tiple, 2009). Butterflies are most beautiful and attractive than most other insects and have fascinated human imagination and creativity. They are valuable pollinators when they fly from plant to plant, gathering nectar and are the one of the important food chain components of the birds, reptiles, spiders and predatory insect. In the present investigation, a checklist of species of order Lepidoptera Orthopteran and Odonates of Lonar Lake has been prepared the lists (Table-1). But order Orthopteran and Odonates diversity has been firstly reported in this study.

MATERIALS AND METHODS

The insect fauna were observed in an around the crater by using transect method. Five lines transects were setup, approximately 500m long and 10m wide. The transect lines were walked at a constant space for approximately half an hour. In Transect were walked from 7.00 am to 11.00 am, when insect are most active. Insects were observed and identified by the guidelines of (Gunathilagaraj et al., 1998, Kunte, 2000). The spot observation was followed by photography binocular with high zoom power and rarely the fauna were collected from site for their identification and after the observation the insect were released in the environment. Similarly the dead insect and their body parts were such as wings, feathers, carapace; butterfly wings were also collected and considered in observation. Butterflies species were identified directly in the field visually with the help of field guide followed by photography and VDO recording.

Statistical analysis

Species diversity was calculated using the Shannon Index, which pools the number of species within a site with the relative abundance of each species (Shannon 1948; Magurran 1988, Odum 1997; Krebs 1989).

$$H' = -\Sigma\, pi \ln pi$$

Here, pi is the proportion of the ith species in the total sample. The number of species (species richness) in the community and their evenness in abundance (or equitability) are the two parameters that define H'.

B. Pielou's Evenness index (Equitability) or J': The species evenness is the relative abundance or proportion of individuals among the species. Evenness of species reveals how their relative abundance is distributed in a particular sample or site (Pielou 1969; Magurran 1988).

$$J' = H' / \ln S$$

Here, S is the number of species present in the site. The value of J' ranges from 0 to 1. The less variation was communities between the species, the higher value of J'.

RESULTS AND DISCUSSION

In present study, order Lepidoptera, Orthoptera and Odonata, 15 species of butterflies were reported out of which 2 species remains unidentified. 13 species identified belonging to 4 families of order Lepidoptera. The family Pieridae, represented by 6 species was the most dominated followed by *Danaidae* (4 species), *Nymphalidae* (2 species) and only 1 species of family *Papilionidae* (Table 1).

n order Odonata and Orthoptra, 3 species of Odonates were observed but 2 species is identified and 1 species is unidentified. These two identified species belonging to Anisoptera i.e. *Orthetrum pruinosum neglectum* and *Orthetrum sabina sabina* (Table 1) and 5 species of orthopterans were identified in 4 families, 1 species of Gryllidae, 1 species of Tettigoniidae, 1 speceis of Catantopidae and 2 species of Acrididae (Table 1). The observed and identifies Orthopteran such as, *Gryllus bimaculatus, Tettigonia viridissima, Patanga japonica, Locusta migratoria* and *Melanoplus bivittatus.* Previous studied on butterflies of Lonar Lake and reported that the 48 species of butterflies under five families. Nymphalidae and Pieridae were found dominant 19 and 15 species respectively, followed by Lycaenidae (9 species), Papilionidae (4 species) and one species from the family Hesperiidae. These were drastic changes and decline the biodiversity due to continuous habitat destruction, deforestation and agricultural spraying Palot and Soniya, (2003).

Table 1: Insect (Arthropod) Diversity of Lonar Lake (Total- F-9; G-17; S-17)

Sr. No.	Order	Family	Genus	Species
1	**Lepidoptera (F- 4; G- 11; S- 13)**	1)Papilionidae	*1)Papilo*	*polytes*
2		2)Pieridae	*2)Cepora*	*nerissa*
3			*3)Ixias*	*pyrene*
4			*4)calotis*	*etrida*
5			*5)Catopsilia*	*pomona*
6			*6)Terias*	*hecabe*
7				*laeta*
8		3)Nymphalidae	*7) Junonia*	*lemonias*
9			*8) Hypolimnas*	*bolina*
10		4)Danaidae	*9) Danaus*	*chrysippus*
11				*genutia*
12			*10) Tirumala*	*limniace*
13			*11) Euploea*	*core*
14	**Odonata (F- 1; G- 1; S- 2)**	1) Libellulidae	1) *Orthetrum*	1)*pruinosum neglectum*
15				2) *Sabina*
16	**Orthoptera (F- 4; G- 5; S- 5)**	1) Gryllidae	1) *Gryllus*	1) *bimaculatus*
17		2) Tettigoniidae	2) *Tettigonia*	2) *viridissima*
18		3) Catantopidae	3) *Patanga*	3) *japonica*
19		4) Acrididae	4) *Locusta*	4) *migratoria*
20			5) Melanoplus	5) bivittatus

(F-Family; G-genus; S-species)

Table 2: Seasonal Dynamics of Lepidoptera Population to their Species Diversity and Dominance

Parameter \ Family	Pieridae	Nymphalidae	Danaidae	Papilionidae
Dominance_D	0.137	0.1207	0.122	0.1201
Shannon_H	2.212	2.286	2.264	2.273
Simpson_1-D	0.863	0.8793	0.878	0.8799
Evenness_e^H/S	0.761	0.8195	0.8018	0.8092
Equitability_ J	0.8901	0.9199	0.9111	0.9148

Table 3: Seasonal Dynamics of Orthoptera and Odaonata Population to their Species Diversity and Dominance

Parameter \ Family	Gryllidae	Tettigoniidae	Catantopidae	Acrididae	Libellulidae
Dominance_D	0.1178	0.1166	0.1154	0.1172	0.1058
Shannon_H	2.25	2.272	2.281	2.263	2.343
Simpson_1-D	0.8822	0.8834	0.8846	0.8828	0.8942
Evenness_ e^H/S	0.7909	0.8079	0.8157	0.8009	0.868
Equitability_ J	0.9056	0.9141	0.918	0.9106	0.943

Abundance profile for Insect (Arthropods) observed in different season in Lonar Lake:

The abundance in population of butterflies has been significantly recorded during all the season or months in Lonar Lake.

Species Diversity and Evenness of different Families of Arthropods:

Species richness i.e. the total number of species encountered, diversity index, and evenness index did not varies among the families (Table 2 and Fig. 1). However, in the Lepidopterans families, Nymphalidae

have the higher values as compare to others (Table 3 and Fig. 2). In order Orthoptera and Odonata, *Catantopidae* and *Libellulidae* has higher values were obtained.

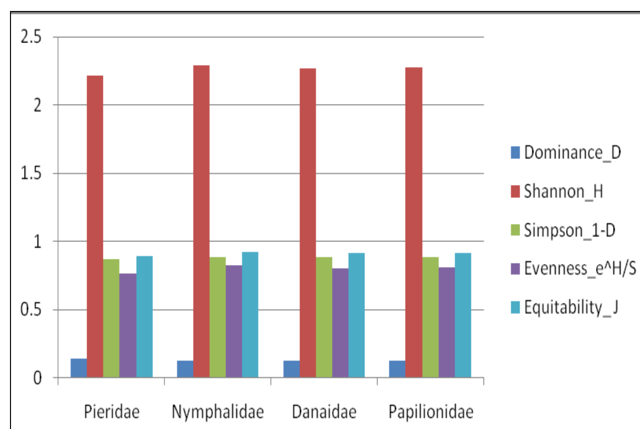


Fig. 1: Diversity and Dominance of Different Families of Order –Lepidoptera

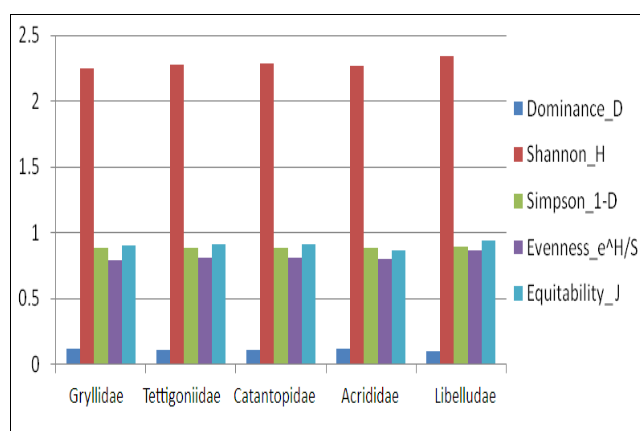


Fig. 2: Diversity and Dominance of Different Families of Order - Orthoptera and Odonata

The entire crater system is under the ecological stress due to disturbance of many ecological parameters in short the ecology of lake is under serious condition due to destruction of natural habitat and heavy deforestation.

REFERENCES

Tiple Ashish D and Khurad Arun M (2009) Butterfly species diversity, Habitats and seasonal distribution in and around Nagpur City, Central India. World Journal Zoology 4 (3): 153-162.

Tiple Ashish D (2011) Butterflies of Vidarbha region, Maharashtra State, central India. Journal of Threatened *Taxa* 3(1): 1469-1477

Battist A (1988) Phytophagous insects in the energy flow of an artificial stand of Pinus nigra Arnold in North Italy. Redia, 71(1): 139-160.

Chakaravarthy AKD (1997) Rajagopal and R. Jagannatha: Insects as bio indicators of conservation in the tropics. *Zoo's Print J*., 12, 21-25.

Gunathilagaraj K, Perumal TNA, Jayaram K and Kumar MG (1998) Some South Indian Butterflies. Nilgiri Wildlife and Environment Association, Tamil Nadu, India. 274pp.

Jana S, Pahari PR, Dutta TK and Bhattacharya T (2009) Diversity and community structure of aquatic insects in a pond in Midnapore town, West Bengal, India. *J. Environ. Biol.*, 30, 283-287.

Krebs C (1989) Ecological Methodology. HarperCollins, New York, 654pp.

Kunte K (2009) Occurrence of *Elymnias obnubila* Marshall and de Nicéville, 1883 (Lepidoptera: Nymphalidae: Satyrinae) in southern Mizoram: Range extension of the species and an addition to the Indian butterfly fauna. Journal of Threatened *Taxa* 1(11): 567-568.

Kunte K (2000) Butterflies of Peninsular India. Indian Academy of Sciences, Universities Press (India) Limited. 254.

Magurran AF (1988) Ecological Diversity and Its Measurements. University Press, Cambridge, 192pp.

May PG (1992) Flower selection and the dynamics of lipid reserves in two nectarivorous butterflies. Ecology, 73: 2181-2191.

Odum EP (1997) Ecology: A Bridge between Science and Society. Sinauer Associated Inc. Sunderland, Massachusetts, USA, 330pp

Palot Muhamed Jafer and Soniya VP (2003) A Preliminary Report on the Butterflies of Lonar Crater Lake, Buldhana District, Maharashtra. *Zoos' Print Journal*, 18 (11): 1267-1268

Pielou EC (1969) An Introduction to Mathematical Ecology. John Wiley, New York, 286pp.

Raup DM (1994) The role of extinction in evolution. Proceedings of the National Academy of Sciences 91 (15): 6758–6763.

Sala Osvaldo E, Meyerson Laura A and Parmesan Camille (2009) Biodiversity change and human health: from ecosystem services to spread of disease. Island Press. 3–5.

Shannon CE (1948) A mathematical theory of communication.Bell System Technical Journal 27: 379-423 & 623-656.

.

© 2016| Published by IJLSCI

RESEARCH ARTICLE

Studies of water borne *E.coli* from the Congo red medium differentiation between pathogenic & non pathogenic in the broiler farms in Madhya Pradesh

Mishra Priti[1], Ther Sandip V[2] , Shukla Satish[2*]

[1]College of Fishery Science, Nanaji Deshmukh Veterinary Science University Jabalpur, (M.P.) India

[2]Central Poultry Diagnostic Laboratory (Phoenix Group) Jabalpur (M.P.) India

*Corresponding author E-mail : skshukla24@gmail.com

Manuscript details:

Received: 25.01.2016
Revised : 05.03.2016
Accepted: 16.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Mishra Priti, Ther Sandip V, Shukla Satish (2016) Studies of water borne *E.coli* from the Congo red medium differentiation between pathogenic & non pathogenic in the broiler farms in Madhya Pradesh. *International J. of Life Sciences*, 4(1): 137-139.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

To aim of this study was to isolate and to identify the pathogenicity of *E. coli* from the water in poultry farms. To conduct this study, water samples were collected from the poultry farms in the vicinity of Jabalpur. Around 200 water samples were collected from the 60 poultry farms, during the period of September 2011 to January 2014. All the water samples were cultured on Mac-Conkey's and EMB agar plates. The positive samples were examined for coliform. confirmation of *E. coli* was based on the morphology of colonies, gram staining and biochemical characters. *In vitro* pathogenicity test for *E. coli* was carried out through the Congo-red binding activity. Out of 200 water samples processed for coliforms, 150 produced growth on Mac-Conkey's agar. Biochemical characteristics identified 66 of these as *E. coli.* Out of 66 isolates of *E. coli,* 40 resulted in the growth of brick red colonies shown the pathogenic. While remaining 26 samples produced colorless colonies after 72 hours of incubation at room temperature and were confirmed as non pathogenic.

Keywords: Water, *E. coli,* Congo Red, Broilers poultry farms, Madhya Pradesh.

INTRODUCTION

Water is regarded as the most essential factor for life, it is not possible to say its exact requirements. Chicken generally take double amount of water compare the amount of feed consumed on a weight basis. During summer season, water requirements increased upto four times. The presence of microorganisms is typically a result of surface contamination by organic materials and can result in poor performance. (Blake ,Hess: 2001). Through the fecal matter, drinking water is contaminated with coliform bacteria (including *E.coli, klebsiella, Enterobacter, Proteus*). As per standard bacterial contaminants should not be present in drinking water and levels maintains should be zero. (http://www.2ca.uky.edu/).

An insufficient amount of water resulting depletion in growth and egg production. Microbes free drinking water has important role in the broiler farming. Contaminated water affects the growth of birds and increases the economic losses to poultry farmer. Contaminated water is the main source of disease spread among the chicken birds. If drinking water is contaminated it can cause different bacterial infections including *Colibacilosis, salmonellosis, staphylococcosis*. So that hygiene of water is utmost important for gaining the good profit by the production and health management in a poultry flock.

E.coli is pathogen causing various diseases in poultry viz: CRD, CCRD, salpingitis, yolk sac infection, air sac disease, perihepatitis, enteritis, omphalitis, colibacillosis etc. (Vegad, 2007).

The objective of present study was carried out to check the presence and pathogenicity of *E. coli* in drinking water at different poultry farms in Jabalpur. Around 200 water samples were collected from the 60 poultry farms, during the period of September 2011 to January 2014.

MATERIALS AND METHODS

To know the presence of *E.coli* in drinking water, we collected 200 water samples from 60 broiler poultry farms in and around the Jabalpur city. Out of these, 125 samples collected directly from the water bore well and 75 from the drinkers and channels placed in poultry shed. The sample of water was not treated with any sanitizers and acidifiers. A water sample of 200 ml was collected directly in sterile screw capped glass bottles after running the tap few minutes. Taken 5 ml water sample was mixed d an aliquot of 50 ml was cultured on Mac-Conkey's broth. These Mac-Conkey's broth samples were streaked on agar plates of on EMB, Mc-Conkey's agar, Tergitol -7 agar, purified lactose fermenting colonies were counted. Picked up for further morphological and biochemical characterization including, Indole, MR, VP, Citrate utilization, Urease activity, oxidase, catalase, motility. (Cowan and Steel,1975; Ewing 1986).

Fermentation of carbohydrates:
Isolate of *E.coli* were confirmed by the carbohydrates fermentation activity: maltose, lactose, sucrose, dulcitol, adonitol, dextrose, xylose, mannitol. Peptone water with phenol red broth used as fermentation indicator. Isolates of *E. coli* were inoculated into tubes in the carbohydrates and kept at 37^{0}C for 24hrs. The positive results shown from red to yellow, while the negative results remained red.

Congo red Binding Assay:
E.coli. positive samples streaked on congo red agar plates to know the pathogenicity of isolates. Described by Berkhoff and Vinal (1986). Rosenberger *et al.* (1985), Kalorey *et al.* (2002), Parul & Bist *et al.* (2014) Panigrahy *et al.* (1990) Sharma, *et al.* (2006). Each isolate were streaked on separate plate and kept at 37^{0}C for 24hrs. After 24hrs incubation, the cultures were kept at room temperature for 48 hours. Pathogenic *E. coli* were identified by Congo red positive isolates produced brick red colonies. The non-pathogenic isolates appeared as colourless after 48 hours in room temperature.

RESULTS AND DISCUSSION

Out of 200 water samples processed for Coliform count, 150 produced growth on Mac Conkey's agar and were confirms as Coliform bacteria. The results of the Congo red binding test indicate that 53.3% of samples of *E.coli* produced Congo red positive (Table 1).

Table 1: Classification of the water samples on the basis of bacterial counts

S. No.	Water samples divide on the basis of Coliform count (CFU/ml)	No. of Total samples	No. of Lactose Fermenters Colonies
1	5-49	38	22
2	50-99	46	28
3	100-250	35	16
4	More than 250	31	11
	Total	**150**	**77**

In between period of this experiments, we also found other bacterial species in the water samples *viz*: *Pseudomonas spp. Salmonella spp., Proteus spp. Enterobacter, Klebsiella, Edwardsiella spp., Citrobacter and Serratia* etc. This study focused on the percentage of *E. coli* in poultry water samples, hence other coliform was neglected. (see Table-1). 45% bind the Congo red dye out of seventy seven samples, and were considered as entero-invasive *E. coli.* This finding is according the result of other workers. Those recommended the use of Congo red dye with the aim of differentiation between pathogenic and non-pathogenic microorganisms (Berkhoff and Vinal, 1986; Stebbins *et al.* 1992).

CONCLUSION

Out of 77 samples 45% bind the Congo red dye and were grouped as pathogenic *E. coli.* It is confers that the occurrence of coliform has main role in farm sanitation and disease management. If drinking water is contaminated it increases the chance of infection of pathogenic *E. coli.* In the presence of other member of *Enterobacteriacae* is insignificant as they are considered to be introduced mainly from soil and sewage source. Also it considered *E. coli* is present in environment.

REFERENCES

Berkhoff HA and Vinal AC (1986) Congo red medium to distinguish between invasive and non invasive *Escherichia coli* pathogenic for poultry. *Avian Dis*., *30*: 117-121.

Blake JP and Hess JB (2001) Evaluating Water Quality for Poultry. The Alabama Co-operative extension system (Alabama A&M University and Auburn University ECP,publication date 10/04/2001. DOI//Access web date 24/12/2015//: http://www.aces.edu/agriculture/ livestock-poultry.

Cowan ST and Steel K (1975) Manual for the Identification of Medical Bacteria, 1st edn., Cambridge University Press, Cambridge, USA.

Ewing WH (1986) Edwards and Ewing's Identification of Enterobacteriacae, 4th edn. *Elsevier Science Publishing Co. Inc., New York*. Pp. 132-134.

http://www.2ca.uky.edu/poultryprofitability/production-manual/chapter12.pdf. Access web . 11.15a.m. 24/12/2015

Kalorey DR, Kurkene NV, Hame SD, Ingle VC, Patil AA, Sakhare PS and Warke SR (2002) Congo red binding ability of *E.coli*. *Indian J. Comp. Microbiol*. *Immunology Infectious disease.* 23:79-80.

Panigrahy B,Yushen L (1990) Differentiation of pathogenic and non pathogenic *Escherichia coli* isolated from poultry. *Avian. Dis*. *34*: 941-943.

Parul Bist B, Sharma B and Jain U (2014) Virulence associated factors and antibiotic sensitivity pattern of *Escherichia coli* isolated from cattle and soil, *Veterinary World 7*(*5*): 369-372.

Rosenberger K, Fries PA, Cloud SS and Wilson RA (1985) *In vitro* and *in vivo* characterization of avian *Escherichia coli.* II. Factors associated with pathogenicity. *Avian Diseases, 29*: 1094-1107.

Sharma KK, Soni SS and Sunil Meharchandani S (2006) Congo red dye agar test as an indicator test for detection of invasive bovine *Escherichia coli. Veterinarski archiv 76* (*4*): 363-366.

Stebbins MB, Berkhoff HA and Corbett WT (1992) Epidemiological studies of Congo Red *Escherichia coli* in broiler chicken. *Canadian Journal of Veterinary Research, 56*: 202-205.

Vegad JL (2007) *Poultry Diseases-A Guide for Farmers and Poultry Professionals.* 2nd edn . International Book Distributing Company, Lucknow, India.

© 2016| Published by IJLSCI

RESEARCH REPORT

Cercarial fauna of Malabar, Kerala- I

Venugopalan Nambiar M

Department of Zoology, Government Brennen College, Dharmadam, Thalassery,Kerala – 670 106, India.
Email: venug282@gmail.com

Manuscript details:

Received: 14.01.2016
Revised : 04.03.2016
Accepted: 22.03.2016
Published : 10.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Venugopalan Nambiar M (2016) Cercarial fauna of Malabar, Kerala- I, *International J. of Life Sciences*, 4(1): 140-144.

Acknowledgement:
The author is thankful to University Grants Commission, New Delhi, India for the financial assistance in the form of a Minor Research Project.

Copyright: © 2016 | Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

Two new species of echinostomecercariae, *Cercaria malabari*I n.sp. found in pilid snails, *Pilavirens* and *Cercaria malabari* II*n. sp*. In *Bellamyadissimilis*, collected from different freshwater bodies of Malabar, Kerala are described. Descriptions of metacercariae are also given.

Keywords: *Cercaria malabari* I n.sp.,*Cercaria malabari* II n.sp., Echinostomecercaría.

INTRODUCTION

The pilid snails, *Pilavirens* and vivipariid snails, *Bellmya dissimilis* were collected from different water bodies, such as paddy fields, ponds, rivulets in Malabar region of Kerala were screened for cercarial study. Two species of echinostome cercariae having 37 collar spines were noticed from each snail species. Detailed studies proved that these cercariae were differ from all the known echinostome cercariae and reported here are *Cercaria malabari* I n.sp. and *Cercaria malabari* II n.sp. respectively after the region of collection of snails.

MATERIALS AND METHOD

Snails were collected from paddy fields, ponds and rivulets of Malabar region of Kerala, and infected snails were isolated and kept for cercarial study. The cercariae emerging from infected snails were studied on their morphology and behaviour. Internal structures were observed using supravital stains, Neutral red and Nile blue sulphate with the aid of Leitz Diaplan Phase-contrast microscope. Permanent preparations were prepared after staining them in Alum Carmine, following the procedure outlined by Cantwell (1981). Measurements were taken from 10% formalin fixed larvae. Measurements are given in micrometers (µm) with mean values in parentheses. Sketches are drawn with the aid of Camera Lucida(prismtype).

RESULT AND DISCUSSION

1. *Cercaria malabari* I n.sp.

Cercariae were found emerging from pilidsnails in moderate during the morning hours. They showed photopositivity and accumulated in large numbers on the brighter side of the container. They exhibited typical wobble-like motion with constant flexion and extension of tail. The cercariae performed leech-like movements on a glass slide with a thin film of water or at the bottom of the container. They remained alive for about 12 h in well-water.

Description (Fig. 1a, b): Body elongate or oval, spinose, with 8 pairs of sensory hairs at anterior half, measured 355-490 (412) x 132-290 (212). Collar distinct, 74-119 (87) wide with 37 spines, 8.5-10.5 (9.8) in length. Spines arrangement characteristic: five corner spines on each ventral lappet, 3 oral and 2 aboral; 6 laterals on each side in single row; 15 dorsals, 8 oral and 7 aboral. Oral sucker roughly oval, measured 44-71 (56) x 41-65 (54). Acetabulum post-equatorial, protrusible, 65-89 (75) in diameter. Tail cylindrical, aspinose, set in a concavity at the posterior end of body, measured 327-550 (42) x 66-93 (70), with 2 dorsal, 2 ventral, 2 ventro-lateral and one ventral finfolds. At anterior half, 12-14 sensory hairs present. Tip of tail finger-like, capable of independent contraction and expansion.

Mouth subterminal, Prepharynx 18-25 (22) long, with a small glandular prepharyngealbody. Pharynx muscular, 31-43 (36) x 17-22 (19) in size. Oesophagus solid, 142-176 (161) long, consisting of 9-10 cells. Intestinal caeca solid, 231-249 (235) long; each caecum composed of 10 cells and an additional cell at the point of bifurcation. Penetration glands 4 pairs, lobate, along oesophagus; gland ducts open on dorsal lip of oral sucker in 2 pairs. Paraoesophageal gland-cells 16-18 in number, with duct outlets around oral sucker and pharynx. Cystogenous glands numerous, densely distributed throughout body, and filled with rod-shaped contents. Genital primordia consist of two masses of cells, one at the pre-acetabular region, the other between acetabulum and base of tail and connected by a string of cells passing dorsal to acetabulum. Excretory system stenostomate; excretory bladder bipartite, at posterior end of body; main collecting ducts distended between collar and acetabulum, each containing 60-90 excretory granules. Caudal excretory duct extends to anterior fifth of tail, and then bifurcates into two lateral branches. Flame cell formula: 2[(3+3+3) + (3+3+3)] = 36.

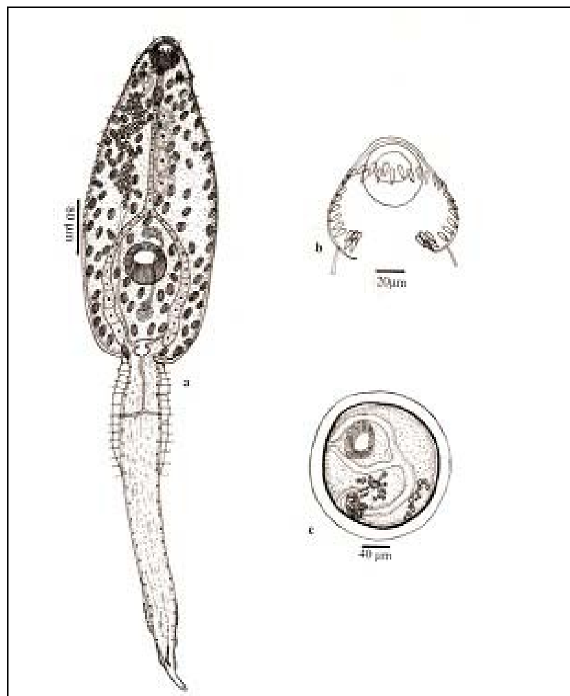


Fig. 1: Cercari malbari I n. sp.
a. Cercari **b.** Collar with spines
c. encysted metacercaria

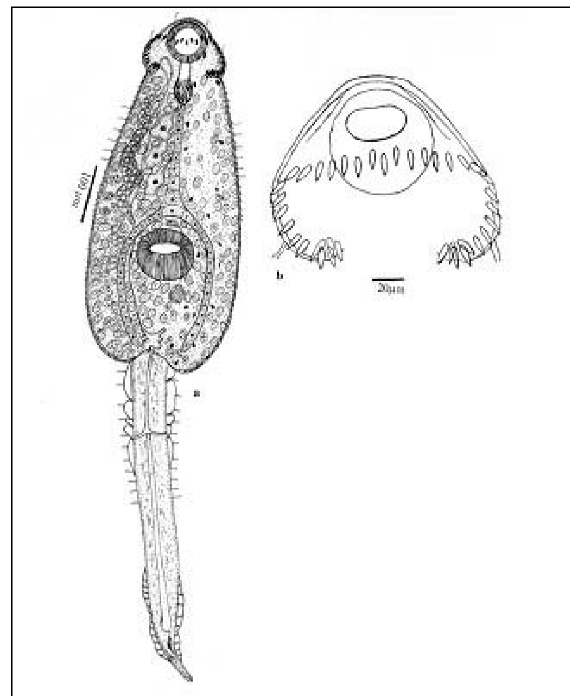


Fig. 2: Cercari malbari II n. sp.
a. Cercari **b.** Collar with spines

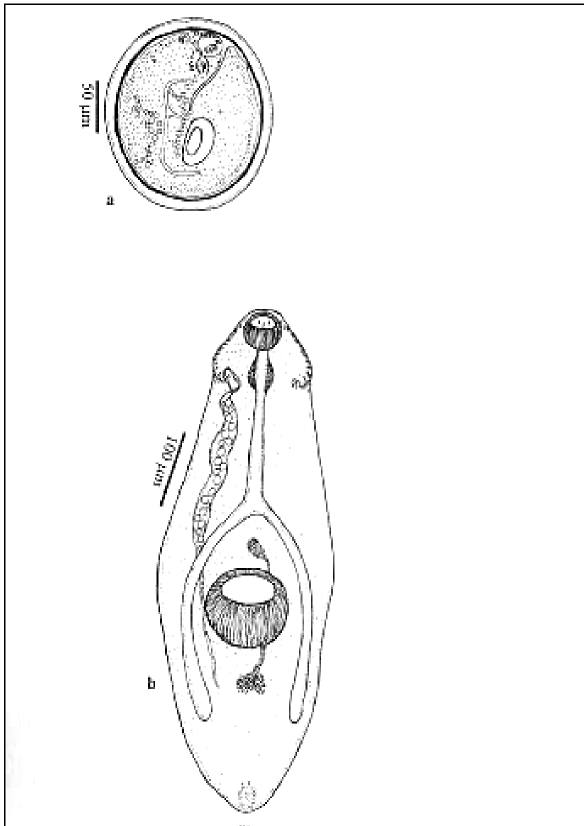


Fig. 2: Cercari malbari II n. sp.
a. encysted metacercaria
b. excysted metacercaria

Metacercaria (Fig 1c): The cercariae encysted in the auricle of *Bellamyadissimilis* and hepatopancreas of *Indoplanor bisexustus* and *Lymnaealuteola.* Occasionally, a few metacercariae were found in the kidney and hepatopancreas of the host snail, *P. virens*. They also encysted on gill arches of tadpoles and fishes. Under experimental conditions, metacercariae were obtained from these hosts. Cyst spherical, 192-204 (198) in diameter. Cyst wall three-layered, 13-15 thick; outer, thin, transparent layer, 0.7-2 thick; inner, translucent layer, 3-4 thick and middle, transparent layer 7-9 thick. The cysts recovered from gill arches of fishes and tadpoles were without the outer layer. Inside the cyst the larva remained curled up. Oral sucker, acetabulum, collar and collar spines, pharynx, oesophagus and caeca visible through cyst wall.

First intermediate host : *Pilavirens*
Locality : Cherukunnu, Payyanur, Pinarayi in Kannur district
Period of collection : 2012-2014
Prevalence : 131 out of 2230 (5.87) snails were examined

Remarks: The present cercaria has a tail identical with that of the cercariae of the genus *Echinostoma*, as described by Fried et.al (1998). The number and arrangement of collar spines can be taken as one of the important distinguishing characters of echinostomecercariae and the present form has 37 collar spines arranged in unique pattern described by Mohandas (1973),Kanev (1994) and Kanev et. al (1995), is compared with other 37 spinned echinostomecercariae, cercariae of *E. echinatum, E. trivolvis, E. cinetorchis, E. rodriguesi and Cercaria unnaoensis*III Pandey and Lal1982, *C. andhraensis* Ganapathi and Rao 1969.

The present cercaria differs from that of cercaria of *E. echinatum* in body spination, number of penetration glands, paraoesophageal gland-cells, cells in the oesophagus and caecum and flame cells and in having a different snail host.
The difference in number of paraoesophageal gland-cells and cells in the oesophagus and caecum make the cercaria of *E. trivolvis* distinct from the present cercaria. Further, the hosts utilized by the cercariae of the two species are different.

The cercariae of *E. cinetorchis*differs from the present cercaria in the arrangement of collar spines, the number of penetration glands, oesophageal and caecal cells, in the nature of cystogenous material and in morphometry. Moreover, the cercaria of *E. cinetorchis* develops in *Hippeutiscantori* whereas that of the present form in *Pilavirens*. In the cercaria of *E. caproni* there are 8 penetration glands, 18 pairs of flame cells, 7 cells each in oesophagus and caecum and in the cercaria of *E. rodriguesi*, the oesophagus and caecum consists of 7 cells, penetration glands are 6 in number and there are 21 pairs of flame cells.

The present cercaria is compared with other 37 spinned echinostome cercariae, the adults of which are not known. Among these, the present form has a tail identical with that of *Cercaria unnaoensis* III Pandey and Lal, 1982. But it stands distinct from the others in one or more of the following characters: distribution of body spines, number of oesophageal and caecal cells, nature of cystogenous material, number and position of penetration glands, number of flame cells, number and disposition of setae, presence or absence of paraoesophageal gland-cells. Again it differs in the snail hosts utilized.

The present cercaria needs comparison with *Cercaria andhraensis,* the only species of echinostome cercaria known from a *Pila*species in India till date. Ganapati and Rao (1969) reported it from *P. globosa* in Waltair. However the presence of 33 collar spines and absence of tail fin folds make it distinctly different from the present form which has 37 collar spines and 7 finfolds on tail. Besides, there are differences in several other characters and in morphometry.

In view of these reasons which differentiate the present echinostome cercariae from the closely related forms, this cercaria is considered new to science and is reported here as *Cercaria malabari* I n.sp after the name of region of collection.

2. *Cercaria malabari*II n.sp.

Cercariae were released by snails throughout day-time. They were found swimming actively for about 6 hours, then sank to the bottom and died several hours later. Cercariae were negatively phototactic, and performed leech-like movements at the bottom of the container.

Description (Fig 2a, b): Body elongate, spinose, measured 431-601 (527) x 227-331 (283), with 8 pairs of sensory hairs in anterior half. Body spines 3-4.5 long in pre-acetabular region and 1.8 to 2.7 in post-acetabular region. Collar distinct, 119-146 (132) wide, armed with 37 spines, 11-13 (12.1) long. Spine arrangement characteristic: five corner spines on each ventral lappet, 3 oral and 2 aboral; 6 laterals on each side in single row; 15 dorsals, 8 oral and 7 aboral. Oral sucker round to oval, measured 62-69 (65) x 69-73 (71). Acetabulum post-equatorial, protrusible, 73-84 (79) in diameter. Tail cylindrical, aspinose, ending in a finger-like projection, capable of independent movement; 354-420 (382) long, 73-85 (82) wide, with 7 finfolds, 2 dorsal, 2 ventral, 2 ventro-lateral and a small ventral just anterior to the finger-like projection. Ten pairs of sensory hairs at anterior half of tail.

Mouth subterminal, Prepharynx 23-35 (28) long; pharynx ovoid, muscular, 31-39 (34) x 39-42 (41) in size. Oesophagus solid, 193-220 (199) long, consisting of 10 cells filled with coarse granular contents. Intestinal caeca solid, 163-293 (236) long, extending to posterior end of body, each caecum with 14 cells and an additional cell at bifurcation.

Penetration glands 3 pairs, located along oesophagus; each gland lobate, with round nucleus and finely granular contents. Paraoesophageal gland-cells at pharyngeal region with 12-14 outlets opening at oral sucker and 6 at pharyngeal zone. Cystogenous glands abundant, distributed throughout body, below the level of prepharynx; filled with round or oval contents.

Genital primordia consist of two cell masses, one anterior to and the other posterior to acetabulum, both connected by a string of cells passing dorsally to acetabulum. The excretory system stenostomate; excretory bladder bipartite, at posterior end of body; main collecting ducts distended between pharyngeal region and acetabulum, each containing 30-60 excretory granules. The caudal excretory duct extents one fourth of tail-length before bifurcating into two lateral branches. Flame cell formula: 2[(3+3+3)+(3+3+3)] = 36.

Metacercaria (Figs. 3a, b): The cercariae encysted in the same snail host which liberated them or in other snails of the locality. Cysts were recovered from the pericardial cavity, kidney and muscles of *Bellamyadissimilis, Lymnaealuteola, Pilavirens, Indoplanorbisexustus* and*Thiaratuberculata*and tadpoles of *Bufobufo*. Metacercarial cysts spherical, 185-191(188) in diameter. Cyst wall double-layered with an outer, transparent, delicate, easily detachable layer of 5-6 thickness and an inner, translucent layer of 2-3 thickness. Inside the cyst the larva remained curled up. Metacercariae exposed to digestive juice of chicks excysted in 3-4 hours. Excystedmetacercariae measured 551-648 (592) x 216-326 (285).

First intermediate host : *Bellamyadissimilis*
Locality : Thalassery and Thazhechovva in Kannur District
Period of collection : 2012-2014
Prevalence : 353 out of 3827 (9.22%) snails examined were infected.

Remarks : The present form of cercaria also has 37 spinned collar and 7 tail finfolds Hence it can be compared with cercariae of genus *Echinostoma* with 37 collar spines, *Echinostomaechinatum, E. trivolvis, E. cinetorchis, E. caproni, E. redriguesi*, and *Cercariaspinosa* Pandey and Singh 1984 and *Cercaria malabari* I n.sp..

The cercaria of *E. echinatum*differs from that of the present form in the number of paraoesophageal gland-cells, penetration glands, flame cells and oesophageal and caecal cells. Cercariae of *E. trivolvis* differ in body spination, number of paraoesophageal gland-cells and caecal cells. Differences observed with cercariae of *E. cinetorchis*in the number of penetration glands, number and arrangement of finfolds, number and distribution of paraoesophageal gland-cells, and in morphometry. The cercaria of *E. caproni* is distinct in the number of penetration glands, body setae, nature of cystogeneous material and number of oesophageal and caecal cells. Besides, the cercaria of *E. rodriguesi* different from the present cercaria in flame cell formula, number of oesophageal and caecal cells and in morphometry.

The present cercaria needs comparison with other cercariae, the adults of which are not known. It differs from *Cercariaspinosa* infecting *Bellamyabengalensis*from Uttar Pradesh in the number and arrangement of tail finfolds on tail and first intermediate host and internal organisation. The present cercaria differs from *Cercaria malabari* I n. sp. in first intermediate host morphometry and morphological features. Therefore, the cercariae under discussion considered new and the name *Cercaria malabari* IIn.sp.

REFERENCES

Cantwell GE (1981) Methods for invertebrates. Invertebrate Staining Procedures (Editor. Clark G) Williams and Wilkins, Baltimore, 255-280

Fried B, Frazer BA and Kanev I (1998) Comparative observations on cercaria and metacercariae of*Echinostomatrivolvis* and *Echinoparyphium* sp. Journal of Parasitology, **84**(3): 623-626.

Ganapati PN and Rao KH (1969) Some observations on the structure and life-history of *Cercariaandhraensis*n.sp. (Trematoda: Echinostomatidae) from the apple snail *Pilaglobosa*Swainson of Waltair. Proceedings of Indian Academy Science, **69**(13): 277-283.

Kanev (1994) Life-cycle, delimitation and redescription of *Echinostomarevolutum* (Frölich, 1802) (Trematoda: Echinostomatidae). Systematic Parasitology, **28**: 125-144.

Kanev I et al. (1995) Redescription of *Echinostomatrivolvis* (Cort, 1914) (Trematoda: Echinostomatidae) with a discussion on its identity. Systematic Parasitology,**32**: 61-70.

Mohandas (1973) Studies on the life history of *Echinostomaivaniosi*n.sp. Journal of Helminthology, **47**: 421-438.

Pandey KC and LalSK (1982) Observation on a new echinostomecercaria. Proceedings of B K TandonCommemorative Lecture Series **1**: 70-74.

Pandey KC and Singh HS (1984) Observations on a new echinostomecercaria. Indian Journal ofHelminthology, **1** (1&2): 45-59.

© 2016| Published by IJLSCI