# Pakistan Journal of Scientific and Industrial Research

**Series B: Biological Sciences** 

Vol. 56, No.1, March-April, 2013



(for on-line access please visit web-site http://www.pjsir.org)

Published by
Scientific Information Centre
Pakistan Council of Scientific and Industrial Research
Karachi, Pakistan

## Pakistan Journal of Scientific and Industrial Research Series B: Biological Sciences

#### **EDITORIAL BOARD**

**Dr. Shoukat Parvez**Editor-in-Chief

Ghulam Qadir Shaikh Executive Editor

#### **MEMBERS**

Dr. T. A. Ajith
Amala Institute of Medical Sciences
Kerala, India
Dr. Christopher Marlowe A. Caipang
Faculty of Biosciences and
Aquaculture, University of Nordland, Norway
Dr. Veronica Leticia Colin
Av. Belgrano y Pasaje Caseros,
Tucuman, Argentina

Prof. E. Miraldi
Pharmaceutical Biology Section
University of Siena, Siena, Italy
Dr. Gunter Muller
Aventis Pharma, Germany
Dr. S. K. Rastogi
Dept. of Chem. & Biochemistry,
Texas State University, USA
Dr. Zafar Saied Saify
ICCBS, University of Karachi,
Karachi, Pakistan

Dr. Hiroshi Shimoda
Oryza Oil & Fat Chemical Co. Ltd.,
Aichi, Japan
Prof. Dr. Toshiyuki Toyosaki
Dept. of Foods and Nutrition
Fukuoka, Japan
Dr. Vasudeo Zambare
Centre for Bioprocessing
Research and Development,
South Dakota, USA

Editors: Shagufta Y. Iqbal Shahida Begum Sajid Ali

Pakistan Journal of Scientific and Industrial Research started in 1958, has been bifurcated in 2011 into:

Series A: Physical Sciences [ISSN 2221-6413 (Print); ISSN 2223-2559 (online)] (appearing as issues of January-February, May-June and September-October) and

Series B: Biological Sciences [ISSN 2221-6421 (Print); ISSN 2223-2567 (online)] (appearing as issues of March-April, July-August and November-December).

Each Series will appear three times in a year.

**This Journal is indexed/abstracted** in Biological Abstracts and Biological Abstracts Reports, Chemical Abstracts, Geo Abstracts, CAB International, BioSciences Information Service, Zoological Record, BIOSIS, NISC, NSDP, Current Contents, CCAB, Rapra Polymer Database, Reviews and Meetings and their CD-ROM counterparts etc.

**Subscription rates (including handling and Air Mail postage):** *Local:* Rs. 2000 per volume, single issue Rs. 350; *Foreign:* US\$ 400 per volume, single issue US\$ 70.

**Electronic format** of this journal is available with: Bell & Howell Information and Learning, 300, North Zeeb Road, P.O. 1346, Ann Arbor, Michigan 48106, U.S.A; Fax.No.313-677-0108; http://www.proquest.com.

**Photocopies of back issues** can be obtained through submission of complete reference to the Executive Editor against the payment of Rs. 25 per page per copy (by Registered Mail) and Rs. 115 per copy (by Courier Service), within Pakistan; US\$ 10 per page per copy (by Registered Mail) and US\$25 per page per copy (by Courier Service), for all other countries.

**Copyrights** of this Journal are reserved; however, limited permission is granted to researchers for making references, and libraries/agencies for abstracting and indexing purposes according to the international practice.

Printed and Published by: PCSIR Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.

#### **Editorial Address**

#### **Executive Editor**

Pakistan Journal of Scientific and Industrial Research, PCSIR Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan Tel: 92-21-34651739-40, 34651741-43; Fax: 92-21-34651738; Web: http://www.pjsir.org, E-mail: info@pjsir.org

## Pakistan Journal of Scientific and Industrial Research Series B: Biological Sciences Vol. 56, No.1, March - April, 2013

## **Contents**

Entomophily, Ornithophily and Anemochory in the Highly Self-incompatible	
Boswellia ovalifoliolata Bal. & Henry (Burseraceae), an Endemic and	
<b>Endangered Medicinal Tree Species</b>	
Aluri Jacob Solomon Raju, Pakkurti Vara Lakshmi and Kunuku Venkata Ramana	1
Prospects of Using Gypsum to Conserve Water and Improve Wheat Yield in Rainfed Aridisols	
Obaid Ur Rehman, Muhammad Rashid, Sarosh Alvi, Rahina Kausar, Rizwan Khalid and Tanvir Iqbal	11
Genetic Divergence Analysis in Pumpkin	
Abul Khair Muhammad Quamruzzaman, Shahabuddin Ahmad, Mohammad Moniruzzaman,	
Mohammad Khalid Jamil and Mohammad Amir Hossain Mollah	18
Poisoning by Plants in the Taza-Al Hoceima-Taounate Region in Morocco	
Toilabiya Loutfia, Hami Hinde, Soulaymani Abdelmajid, Rhalem Naima, Ouammi Lahcen	
Benali Doha, Mokhtari Adbelrhani and Soulaymani-Bencheikh Rachida	23
High-Performance Liquid Chromatographic Quantification of Rifampicin	
in Human Plasma: Method for Therapeutic Drug Monitoring	
Trabelsi Sameh, Eljebari Hanene, Charfi Rim, Salouage Issam, Gaïes Emna,	
Nadia Jebabli, Lakhal Mohamed and Klouz Anis	29
Determination of Limonin and Nomilin Contents in Different Citrus Cultivars	
Using High Performance Liquid Chromatography	
Hazrat Bilal, Waseem Akram, Soaib Ali Hassan, Sumrin Sahar and Muhammad Munir Iqbal	36
Susceptibility of Multidrug Resistant Enterotoxigenic Escherichia coli	
to Saponin Extract from Phyllanthus niruri	
Victor Adeyinka Ajibade and Oladiran Famurewa	41
Heavy Metals Contamination in Fish and Shrimp from Coastal Regions	
of Karachi, Pakistan	
Syed Sanower Ali, Ishratullah Siddiqui, Farooq Ahmed Khan and Alia Bano Munshi	46
Study of Human Cystic Echinococcosis in Hyderabad, Pakistan	
Lochi Ghulam Murtaza, Muhammad Ghiasuddin Shah, Amjad Hussain Merani	
Muhammad Shoaib Khan, Sheeraz Mustafa Khushk and Abdul Manan Khokhar	53

### **Short Communication**

Occurrence of *Staphylococcus aureus* in Milk Based Sweet Products Consumed in Karachi, Pakistan Korish Hasnain Sahir, Zulfiqar Ali Mirani, Muhamamd Naseem Khan, Shagufta Naz and Seema Ismat Khan

**56** 

## Entomophily, Ornithophily and Anemochory in the Highly Self-incompatible Boswellia ovalifoliolata Bal. & Henry (Burseraceae), an Endemic and Endangered Medicinal Tree Species

#### Aluri Jacob Solomon Raju\*, Pakkurti Vara Lakshmi and Kunuku Venkata Ramana

Department of Environmental Sciences, Andhra University, Visakhapatnam 530 003, India

(received September 14, 2010; revised January 16, 2012; accepted February 25, 2012)

Abstract. Boswellia ovalifoliolata (Burseraceae) has been investigated for its reproductive biology. Its flowering, fruiting and seed dispersal events occur in leafless state during dry season. The flowers are small, bisexual, mildly odoriferous and actinomorphic; weakly protandrous but strictly self-incompatible. Insects and sunbirds pollinate the flowers but floral characteristics suggest that entomophily is the principal mode. Bud and flower feeding by a weevil and flower and fruit feeding by the Palm Squirrel have been found to be greatly affecting the success of sexual reproduction. The garden lizard serves as a predator of pollinating insects, especially bees and wasps; while acting so it affects the pollination rate in this tree species. Fruit set in open-pollination is below 10% while it is up to 34% in manual cross-pollination. The study suggests that limitation of cross-pollination, space constraint for seed production from all ovules of the flower and availability of limited resources to the tree with rocky, dry and limited litter in the floor of the forest seem to be the constraints for higher fruit set. Mature fruits dehisce and disseminate their light weight, papery and winged seeds with the aid of wind. The study site being windy provides the necessary driving force for effective dispersal of seeds away from parental trees. Seed germination occurs following rainfall but the growth and development of seedlings depends on soil water and nutritional status. Field observations indicate that the success rate of seedling recruitment is highly limited and it could be due to nutrient-poor soil and water stress resulting from dry spells during rainy season.

Keywords: Boswellia ovalifoliolata, self-incompatibility, entomophily, ornithophily, anemochory

# Prospects of Using Gypsum to Conserve Water and Improve Wheat Yield in Rainfed Aridisols

## Obaid Ur Rehman<sup>a</sup>, Muhammad Rashid<sup>a\*</sup>, Sarosh Alvi<sup>b</sup>, Rahina Kausar<sup>a</sup>, Rizwan Khalid<sup>b</sup> and Tanvir Iqbal<sup>c</sup>

<sup>a</sup>Soil and Water Conservation Research Station Fateh Jang (Attock), Pakistan

<sup>b</sup>Soil and Water Testing Laboratory, Rawalpindi, Pakistan

<sup>c</sup>PMAS-Arid Agriculture University, Rawalpindi, Pakistan

(received August 30, 2011; revised November 19, 2012; accepted November 27, 2012)

**Abstract.** A long term field study (2005-10) was carried out at the farmer's field to evaluate the residual effect of gypsum on soil moisture conservation and wheat production under rainfed conditions. The treatments included application of gypsum @ 0, 1 and 2 t/ha, and its comparison with farmer practice (F.P). The basal dose of fertilizers NPK was applied @ 120, 80, 60 kg/ha every year before the sowing of wheat. Soil samples were collected before the sowing of wheat every year for soil moisture determination. Gypsum application not only resulted in an increase in soil moisture contents (up to 41%) but also improved grain yield up to 23% and straw yield up to 19%. The effect of gypsum was noticeable during low rainfall years compared to control (0 t/ha gypsum). The results showed that the gypsum could be an effective additive for soil amendment, enhancing wheat yield in rainfed areas by conserving soil moisture and nutrients.

**Keywords:** gypsum, wheat, moisture, rainfed, soil moisturte

### **Genetic Divergence Analysis in Pumpkin**

## Abul Khair Muhammad Quamruzzaman<sup>a</sup>\*, Shahabuddin Ahmad, Mohammad Moniruzzaman<sup>b</sup>, Mohammad Khalid Jamil<sup>c</sup> and Mohammad Amir Hossain Mollah<sup>d</sup>

<sup>a</sup>Olericulture Division, Horticulture Research Centre, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh

<sup>b</sup>Physiology Section, Horticulture Research Centre, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh

<sup>c</sup>Training and Communication Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh

<sup>d</sup>Uniconsult International Ltd., Building-1, Sector-6, Road no.-16, Uttara, Dhaka-1230, Bangladesh

(received June 9, 2011; revised January 5, 2012; accepted January 30, 2012)

**Abstract**. Genetic divergence among 18 pumpkin genotypes was estimated using Mahalanobis's  $D^2$  statistic. Altogether four clusters were formed where cluster I contained the highest number of genotypes (8) and cluster II contained the lowest (1). The highest intra-cluster distance was observed for cluster I (0.831) and the lowest for cluster IV (0.651). The highest inter-cluster distance was observed between cluster I and II (24.346). Cluster II recorded the highest mean for fruit number/plant, TSS, fruit yield and minimum in cavity length and cavity diameter. Cluster III had the second highest mean for fruit diameter, fruit number/plant, individual fruit weight, fruit yield and the fewest number of days to  $1^{st}$  female flowering, earliness being a desirable trait. These crosses may produce new recombinants with desirable traits.

**Keywords:** genetic diversity, pumpkin, cluster analysis

## Poisoning by Plants in the Taza-Al Hoceima-Taounate Region in Morocco

Toilabiya Loutfia<sup>a</sup>\*, Hami Hinde<sup>a</sup>, Soulaymani Abdelmajid<sup>a</sup>, Rhalem Naima<sup>ab</sup>, Ouammi Lahcen<sup>b</sup>, Benali Doha<sup>a</sup>, Mokhtari Adbelrhani<sup>a</sup> and Soulaymani-Bencheikh Rachida<sup>bc</sup>

<sup>a</sup>Laboratory of Genetic and Biometry, Faculty of Sciences, Ibn Tofail University, Kénitra, Morocco

<sup>b</sup>Poison Control Centre of Morocco, Rabat, Morocco

<sup>c</sup>Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco

(received September 20, 2011; revised September 7, 2012; accepted September 12, 2012)

**Abstract.** A descriptive retrospective-study was carried out on poisoning cases by plants in Taza-Al Hoceima-Taounate region in Morocco, reported between 1984 and 2007 to the Moroccan Poison Control Centre. During this period, 104 cases of poisoning were recorded. *Atractylis gummifera* was the most incriminated plant. The average age of poisoned patients was  $11\pm7.8$  years. The sex ratio (M/F) was 0.9. According to the data, poisoning by plants was primarily responsible for gastrointestinal system disorders, nervous system attacks, visual disorders and heart failure. A lethality rate of 33.65% was recorded, with a total of thirty-five deaths.

Keywords: poisoning plants, Atractylis gummifera, Morocco, lethal effect

# High-Performance Liquid Chromatographic Quantification of Rifampicin in Human Plasma: Method for Therapeutic Drug Monitoring

Trabelsi Sameh<sup>ab</sup>, Eljebari Hanene<sup>a\*</sup>, Charfi Rim<sup>ab</sup>, Salouage Issam<sup>ab</sup>, Gaïes Emna<sup>ab</sup>, Nadia Jebabli<sup>a</sup>, Lakhal Mohamed<sup>ab</sup> and Klouz Anis<sup>ab</sup>

<sup>a</sup>Laboratory of Clinical Pharmacology, National Centre of Pharmacovigilance,
 9, Avenue Dr. Zouheir Essafi, 1006, Tunis, Tunisia
 <sup>b</sup>Faculty of Medicine, El Manar University, Tunis, Tunisia

(received January 11, 2012; revised March 28, 2012; accepted June 11, 2012)

Abstract. A high performance liquid chromatography (HPLC) method has been developed that allows quantification of Rifampicin in human plasma. The method is based on the precipitation of proteins in human plasma with methanol. Optimal assay conditions were found with a C18 column and a simple mobile phase consisting of 0.05 M dipotassic hydrogen phosphate buffer and acetonitrile (53/47, V/V) with 0.086 % diethylamin, pH = 4.46. The flow-rate was 0.6 mL/min and the drug was monitored at 340 nm. Results from the HPLC analyses showed that the assay method is linear in the concentration range of 1-40  $\mu$ g/mL ( $r^2 > 0.99$ ). The limit of quantification and limit of detection of Rifampicin were 0.632  $\mu$ g/mL and 0.208  $\mu$ g/mL, respectively. Intraday and interday coefficient of variation and bias were below 10% for all samples, suggesting good precision and accuracy of the method. Recoveries were greater than 90% in a plasma sample volume of 100  $\mu$ L. The method is being successfully applied to therapeutic drug monitoring of Rifampicin in plasma samples of tuberculosis and staphylococcal infections patients.

Keywords: rifampicin, liquid chromatography, therapeutic drug monitoring

## Determination of Limonin and Nomilin Contents in Different Citrus Cultivars Using High Performance Liquid Chromatography

## Hazrat Bilal<sup>a\*</sup>, Waseem Akram<sup>b</sup>, Soaib Ali Hassan<sup>a</sup>, Sumrin Sahar<sup>a</sup> and Muhammad Munir Iqbal<sup>c</sup>

<sup>a</sup>Health Services Academy, Opposite NIH, Chak Shahzad, Islamabad, Pakistan <sup>b</sup>Department of Agri-Entomology, University of Agriculture Faisalabad, Pakistan <sup>c</sup>National Institute for Genomic and Advance Biotechnology, NARC, Islamabad, Pakistan

(received April 5, 2012; revised October 31, 2012; accepted November 18, 2012)

**Abstract.** High performance liquid chromatography (HPLC) analysis was done to quantify the amount of limonoids (limonin and nomilin) in seven selected citrus cultivars. According to the HPLC analysis red blood orange (*Citrus sinensis* var *red blood orange*) had maximum amount of limonin (479.77 μg/mL), while rough lemon (*Citrus jambhiri*) had no limonin content. In case of nomilin, rough lemon (*Citrus jambhiri*) had maximum amount of nomilin (54.23 μg/mL), while succari (*Citrus sinensis* var *succari*) had very low amount of nomilin (0.37 μg/mL).

Keywords: limonin, nomilin, HPLC, citrus fruits

## Susceptibility of Multidrug Resistant Enterotoxigenic Escherichia coli to Saponin Extract from Phyllanthus niruri

#### Victor Adeyinka Ajibade<sup>a\*</sup> and Oladiran Famurewa<sup>bc</sup>

<sup>a</sup>The Microbiology Unit, Department of Science Technology, Federal Polytechnic, PMB 5351, Ado-Ekiti, Ekiti-State, Nigeria

<sup>b</sup>Department of Microbiology, Ekiti State University, PMB 5363, Ado-Ekiti, 360101, Nigeria <sup>c</sup>College of Science, Engineering and Technology, Osun-State University, PMB 4494, Oke-Baale, Oshogbo 230212, Nigeria

(received January 31, 2012; revised June 6, 2012; accepted June 7, 2012)

**Abstract.** *Escherichia coli* were isolated from 140 samples of blood, urine, stool and water made up of 15.7%, 42.9%, 30.0% and 25.7%, respectively. From the samples, 71.9% enterotoxigenic *E. coli* (ETEC), 14.3% enteropathogenic *E. coli* (EPEC), 7.1% enterohemorrhagic *E. coli* (EHEC) and 7.1% enteroinvasive *E. coli* (EIEC) occurred as diarrheagenic *E. coli*. Of the ETEC (240) isolates tested for susceptibility to eight conventional antibiotics, 110 (46.0%) showed resistance to all the tested antimicrobial agents. However, of the resistant strains; 24 (22.0%) were multidrug resistant. These were tested against 3.0 mg/mL of saponin extract from *Phyllanthus niruri* and 13 (55.0%) of these were susceptible to the saponin. The antimicrobial activities of saponin from *P. niruri* are of interest since the crude extract was effective at concentration of 3.0 mg/mL to multiple resistant isolates of ETEC.

Keywords: diarrheogenic E. coli, multidrug resistant, E. coli pathotypes, saponin, Phyllanthus niruri

# Heavy Metals Contamination in Fish and Shrimp from Coastal Regions of Karachi, Pakistan

Syed Sanower Alia\*, Ishratullah Siddiquib, Farooq Ahmed Khanband Alia Bano Munshib

<sup>a</sup>Department of Community Health Sciences, Ziauddin University, Karachi, Pakistan <sup>b</sup>PCSIR Laboratories Complex, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan

(received April 26, 2012; revised October 11, 2012; accepted November 13, 2012)

**Abstract.** In the present study, the heavy metals (Pb, Cu, Cd, Fe, Zn) concentration was determined by using atomic absorption spectrophotometer (AAS) in 5 species of fish and 3 species of shrimp commonly taken by locals at the coastal regions of Karachi, Pakistan Concentrations of Cd and Pb studied in tissues of Mushka (*Otolithes ruber*, 0.120 and 1.018 μg/g wet weight) and *Palaemon longirostris* shrimp (2.457 and 0.480 μg/g wet weight) were found near to safe level for human consumption. Mullet, Tarli, Surmai, Dohtar fishes and Blacktiger shrimp were found contaminated by Cd and Pb but still within the limits fit for and human consumption. The distribution of trace metals detected in all fish and shrimp species followed the order of Zn >Pb> Fe>Cu> Cd and Cd> Fe > Zn> Cu>Pb, respectively. Metal concentration exhibited significant species variation and followed the order in fishes as *Otolithes ruber> Liza vaigiensis>Sardinella albella >Scomberomorus guttatus>Pomadasys olivaecum* and in shrimp as *Palaemon longirostris>Penaeus monodon>Penaeus penicillatu*.

Keywords: heavy metals, fish, shrimp, tissues, Karachi coast

### Study of Human Cystic Echinococcosis in Hyderabad, Pakistan

Lochi Ghulam Murtaza<sup>a</sup>, Muhammad Ghiasuddin Shah<sup>a</sup>, Amjad Hussain Merani<sup>a</sup>,
Muhammad Shoaib Khan<sup>b\*</sup>, Sheeraz Mustafa Khushk<sup>a</sup> and Abdul Manan Khokhar<sup>a</sup>

<sup>a</sup>Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan

<sup>b</sup>Gomal College of Veterinary Sciences, Gomal University, D.I. Khan, Pakistan

(received January 31, 2012; revised April 4, 2012; accepted June 21, 2012)

**Abstract.** In order to determine the zoonotic importance of cystic echinococcosis (CE) problem in the humans, a study was conducted of patients record available at the Department of Histopathology, Liaquat University of Medical and Health Sciences, Jamshoro. Data was recorded from 1998 to 2008 by using pre-designed proforma. During that period 44 cases of hydatid cysts were recorded out of 43656 registered cases. Highest prevalence of hydatid cysts was recorded in 1999 (0.21%) and the lowest in 2002 (0.02%), whereas no case was registered in 2003 (0%), respectively. Cystic echinococcosis was more efficiently recorded in females (68.18%) as compared to males (31.81%). To calculate the age wise CE in the population; the total population was divided into four age groups **A** (5-20 years), **B** (21-40 years), **C** (41-60 years) and **D** (61 years - onward). The occurrence of CE was highest recorded in age group **B** (50%) and lowest in-group **D** (4.54%).

Keywords: cystic echinococcossis, Zoonotic disease, dogs

#### **Short Communication**

## Occurrence of *Staphylococcus aureus* in Milk Based Sweet Products Consumed in Karachi, Pakistan

#### Korish Hasnain Sahir, Zulfiqar Ali Mirani\*, Muhamamd Naseem Khan, Shagufta Naz and Seema Ismat Khan

Microbiological Analytical Centre, PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui Karachi - 75280, Pakistan

(received June 13, 2011; revised July 31, 2012; accepted September 26, 2012)

**Abstract.**A total of 290 samples of milk-based products of Khoya (75), Qalaqand (75), Rabri (65) and Rusmalai (75) were tested for occurrence of Staphylococcus aureus. The total staphylococcal counts ranged from  $1.0 \times 10^3$  to  $5.7 \times 10^4$  cfu/g,  $1.0 \times 10^2$  to  $6.0 \times 10^3$  cfu/g,  $1.0 \times 10^3$  to  $3.1 \times 10^4$  cfu/g and  $1.3 \times 10^3$  to  $4.0 \times 10^3$  cfu/g in Khoya, Qalaqand, Rabri and Rusmalai samples, respectively. All the samples tested were found contaminated with staphylococci, however, the percentage of S. aureus was low as compared to coagulase -ve staphylococci. Only 6.7% of Rusmalai samples (only 1.72% of all the samples tested) were found free of coagulase +ve S. aureus. About 8% of Khoya (6 out of 75), 28% of Qalaqand (21out of 75), and 40% of Rabri (26 out of 65) samples were found to be hazardous for human health as they contained coagulase +ve S. aureus whereas, none of the Rusmalai samples was found contaminated with coagulase +ve S. aureus.

Keywords: Staphylococcus aureus, Staphylococci, khoya, qalaqand, rabri, rusmalai