

Pakistan Journal of Scientific and Industrial Research

Series B: Biological Sciences

Vol. 57, No.1, March-April, 2014



(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. Kaniz Fizza Azhar
Executive Editor

MEMBERS

Prof. Z. Adamek
Faculty of Agriculture
Univ. South Bohemia, Czech Republic
Dr. T. A. Ajith
Amala Institute of Medical Science
Kerala, India
Dr. S. A. Barlas
Environ. Protection Division
Penticton, BC V0H 1Z4, Canada
Dr. C. M. A. Caipang
Bio Vivo Technologies As, Norway
Dr. D. Das
C. B. Fleet Company, Inc., VA, USA
Dr. W. Gao
Institute of Environ. and Human Health
Texas Tech. University, USA

Prof. M. J. Jaskani
Plant Tissue Culture Cell
Univ. of Agric., Faisalabad, Pakistan
Dr. A. Khanum
Dept. Biochemistry
Pir Mehr Ali Shah Agric. University
Rawalpindi, Pakistan
Dr. S. Mathews
Pharmacology, The University of
Sydney, NSW, Australia
Prof. E. Miraldi
Pharmaceutical Biology Section
University of Siena, Siena, Italy
Dr. G. Müller
Sanofi-Aventis Pharma
Frankfurt, Germany

Dr. S. K. Rastogi
Dept. of Chem. & Biochemistry
Texas State University, USA
Dr. J. S. Sangha
Dalhousie University Truro,
NS B2N 5E3, Canada
Dr. H. Shimoda
Oryza Oil & Fat Chemical Co. Ltd.,
Aichi, Japan
Prof. Dr. T. Toyosaki
Dept. of Foods and Nutrition
Fukuoka, Japan
Dr. V. Zambare
Centre for Bioprocessing
Research and Development,
South Dakota, USA

Editors: Ghulam Qadir Shaikh 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: ProQuest Information and Learning, 789 E. Eisenhower Parkway, P.O. Box 1346, Ann Arbor, MI 48106-1346, U.S.A.; Fax.No.+1.734.997.4268; <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. 57, No. 1, March - April, 2014

Contents

Effect of Different Levels of Foliar Application of Potassium on Hysun-33 and Ausigold-4 Sunflower (<i>Helianthus annuus</i> L.) Cultivars under Salt Stress Muhammad Arshadullah, Arshad Ali, Syed Ishtiaq Hyder, Imdad Ali Mahmood and Bdar-uz-Zaman	1
Growth and Yield Response of Sunflower (<i>Helianthus annuus</i> L.) to Sulphur and Boron Application Muhammad Tahir, Shahzad Ahmed Shah, Muhammad Ayub, Asif Tanveer and Haseeb-ur-Rehman	5
<i>In vitro</i> Antifungal Activities of Extracts of Fruits and other Morphological Parts of <i>Xanthium strumarium</i> Against the Plant Pathogen, <i>Rhizoctonia solani</i> Nour Ahmed Osman, Samia Elias Alsiddeeg, Nafeesa Elmahi Ahmed and Salah Ahmed Ali Elhussein	11
Effect of Plant Age on Cotton Leaf Curl Disease (CLCuD) in Relation to Environmental Conditions Tariq Mahmood, Muhammad Tahir, Hafiz Tariq Mahmood, Sabahat Hussain and Dil Baugh Muhammad	18
Enrichment of Soymeal Medium to Increase the Rapamycin Production by <i>Streptomyces hygroscopicus</i> Abdel-Hamid Ali Hamdy, Essam Mohamed Ahmed, Lotfy Abd El-Raouf Sallam and Mohamed Abdelaziz Mohamed	25
Stability of Microbial and Chemical Indicators of the Minced Beef Meat under Freezing and Refrigerated Temperature Fahad M. Al-Jasass	32
Comparison of Antibacterial Activity of Crude Alkaloid and Saponin Extract from <i>Phyllanthus niruri</i> Victor Adeyinka Ajibade	41
Utilization of Poultry Excreta for High Density Production of <i>Daphnia carinata</i> (King 1853): Cost Effective and Environmental Friendly Technique Mehrajuddin War and Kareem Altaff	46

Short Communications

- Heat Processing and Cold Storage Effects on Vitamins B₁ and B₂ of Buffalo Milk**
Alim-un-Nisa, Abdul Majeed Sularya, Sajila Hina and Shahid Masood 51
- Biochemical Composition of Koi (*Anabas testudineus*) Collected from Paddy Field of Mymensingh, Bangladesh**
Mohajira Begum and Maruf Hossain Minar 54
- First Record of *Cyphonotus testaceus* (Pallas, 1781) (Coleoptera: Scarabaeidae: Melolonthinae: Melolonthini) for Pakistan**
Zubair Ahmed, Aleš Bezděk and Muhammad Atique Akhter 57

Effect of Different Levels of Foliar Application of Potassium on Hysun-33 and Ausigold-4 Sunflower (*Helianthus annuus* L.) Cultivars under Salt Stress

Muhammad Arshadullah*, Arshad Ali, Syed Ishtiaq Hyder,
Imdad Ali Mahmood and Bdarr-uz-Zaman

Land Resources Research Institute, National Agricultural Research Centre, Islamabad-45500, Pakistan

(received March 20, 2012; revised May 8, 2013; accepted May 27, 2013)

Abstract. A hydroponic study was conducted to see the growth response of two cultivars of sunflower (Hysun-33 and Ausigold-4) to K^+ nutrition under salt stress during the growing season 2011, at National Agriculture Research Centre, Islamabad, Pakistan. Nursery of *Helianthus annuus* was raised in sand and ten-day old seedlings per hole were transplanted in each pot having four holes per pot lid. Half strength Hoagland's nutrient solution was filled in each pot. After the establishment of seedlings, salt stress (6 dS/m) was developed artificially. The treatments were, control, 2 and 4 % K^+ as K_2SO_4 foliar applications. Salt present in the growing medium caused a significant ($P<0.001$), reduction in fresh and dry weights of sunflower. Salt stress suppresses the K uptake from pot. Application of varying levels of K_2SO_4 improved the fresh and dry weights of sunflower under both control and saline conditions. However, the highest increase in fresh and dry weight of control and stressed plants was observed when 2% K was applied. Further increase in the level of K application did not improve fresh and dry weights of salt stress and unstressed plants. The growth medium salts reduced sunflower growth.

Keywords: *Helianthus annuus*; salinity; K foliar application; biomass

Growth and Yield Response of Sunflower (*Helianthus annuus* L.) to Sulphur and Boron Application

Muhammad Tahir, Shahzad Ahmed Shah, Muhammad Ayub, Asif Tanveer and Haseeb-ur- Rehman*

Department of Agronomy, University of Agriculture, Faisalabad, Pakistan

(received February 7, 2013; revised July 31, 2013; accepted September 27, 2013)

Abstract. An experiment was conducted to study the growth and yield response of sunflower to sulphur and boron application. Sulphur (control, 15, 30 kg/ha) and foliar sprays of 1% boron solution (control, spray at 4 week after emergence, 20 days after first spray, at 4 weeks after germination + 20 days after first spray) was applied in soil. The results showed significant increase of all growth and yield parameters by varying levels of sulphur and boron application. Application of 30 kg sulphur/ha enhanced stem diameter (1.99 cm), number of achene per head (765.75) and oil content (36.42%). In case of combined use of sulphur and boron application, maximum plant height (171 cm), head diameter (20.71 cm), 1000-achene weight (54.56 g), biological yield (16.49 t/ha) and achene yield (3.99 t/ha) was recorded by the application of 2 sprays of boron solution (1%) at 4 weeks after germination and reproductive stage.

Keywords: sunflower, sulphur, boron, growth, yield

In vitro* Antifungal Activities of Extracts of Fruits and other Morphological Parts of *Xanthium strumarium* Against the Plant Pathogen, *Rhizoctonia solani

**Nour Ahmed Osman^a, Samia Elias Alsiddeeg^b, Nafeesa Elmahi Ahmed^c
and Salah Ahmed Ali Elhussein^{*d}**

^aUniversity College at Alwajh, University of Tabuk, Saudi Arabia

^bNational Oilseed Processing Research Institute (NOPRI), University of Gezira, Wad Medani, Sudan

^cPlant Pathology Department, Agriculture Research & Technology Corporation, Wad Medani, Sudan

^dFaculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, Kuantan, Malaysia

(received January 16, 2013; revised May 22, 2013; accepted June 27, 2013)

Abstract. *In vitro* antifungal activity of different plant parts of *Xanthium strumarium* (Compositae) was investigated against *Rhizoctonia solani* to seek safe natural alternatives to the harmful synthetic fungicides. The most active plant parts of *X. strumarium* were seeds, extracted with *n*-hexane and the leaves, extracted with absolute ethanol. The two treatments resulted in growth inhibition diameters of 45 mm and 47 mm, respectively. The value of MIC lied between 350.0 and 175.0 µg of *Xanthium* oil/mL. Gas liquid chromatography of the seed oil of *X. strumarium* revealed the presence of the usual fatty acids, palmitoleic (7.6%), oleic (21.6%) and linoleic (70.4%). The oil was separated into free fatty acids fraction and unsaponifiable matter fraction. The unsaponifiable matter fraction was separated on TLC, out of six separated compounds, two were active against *R. solani*. The infrared spectra (FTIR) of these two purified compounds pointed to a long chain hydrocarbon back-bone for both, one of them possessing in addition, an alcoholic moiety.

Keywords: *Xanthium strumarium*, antifungal activity, *Rhizoctonia solani*, seed-oil, unsaponifiable matter fraction

Effect of Plant Age on Cotton Leaf Curl Disease (CLCuD) in Relation to Environmental Conditions

Tariq Mahmood, Muhammad Tahir*, Hafiz Tariq Mahmood, Sabahat Hussain and Dil Baugh Muhammad

Central Cotton Research Institute, Old Shujabad Road, Multan, Pakistan

(received July 9, 2012; revised July 19, 2013; accepted August 20, 2013)

Abstract. The effect of cotton leaf curl disease (CLCuD) and weather variables were studied using same genotypes of cotton, planted at 15 days interval during 15th, 23rd standard weeks of 2010-2011. On an average basis, the 15th standard week planting showed, significantly, less disease incidence than all other sowing dates. The incidence increased as the sowing was delayed up to 23rd standard weeks. Among the sowing dates, regardless of genotypes, disease incidence differed, significantly. The CLCuD boost up during 25th to 29th standard (2010) and 27th to 31st standard (2011) weeks of the year, regardless of sowing date and genotypes. Disease incidence was low during 2011 as compared to 2010. The disease increased sharply during 2010 and gradually during 2011. Average maximum (34.8~39.8 °C), minimum (27.7~28.9 °C) temperature and relative humidity (62~79%) favoured CLCuD progression.

Keywords: cotton leaf curl disease, cotton cultivars, relative humidity, temperature

Enrichment of Soymeal Medium to Increase the Rapamycin Production by *Streptomyces hygroscopicus*

Abdel-Hamid Ali Hamdy, Essam Mohamed Ahmed*, Lotfy Abd El-Raouf Sallam and Mohamed Abdelaziz Mohamed

Natural and Microbial Products Department, National Research Center, Dokki, Cairo, Egypt

(received April 11, 2013; revised October 29, 2013; accepted October 31, 2013)

Abstract. Research was carried out to study the improved and increased production of rapamycin by *Streptomyces hygroscopicus* with soymeal enriched media. Media containing soymeal produced rapamycin upto 82.89 mg/L. The medium was enriched with different additives that can interfere with biosynthesis process. L-tyrosine supplementations led to noticeable increase in the rapamycin production to 112 mg/L. However, the progress was achieved upon addition of the shikimic acid (precursor rapamycin moiety), where, it reached 160 mg/L. The greatest increase was recorded after addition of calcium superphosphate (CaP) and the production achieved 176 mg/L. Other substances like vitamins and trace elements had either no or negative effects on the biosynthesis of rapamycin. The study also showed the ability of low concentrations of calcium phosphate to replace the expensive large amount of shikimic acid.

Keywords: *Streptomyces hygroscopicus*, rapamycin, immunosuppressants, soymeal

Stability of Microbial and Chemical Indicators of the Minced Beef Meat under Freezing and Refrigerated Temperature

Fahad M. Al-Jasass

King Abdul-Aziz City for Science and Technology, National Center for Agriculture Technologies,
P.O. Box 6086, Riyadh 11442, Saudi Arabia

(received January 7, 2013; revised September 26, 2013; accepted October 14, 2013)

Abstract. The microbial spoilage and chemical changes in minced beef meat were monitored during storage at freezing and refrigerating temperatures. Total viable count of *Pseudomonas*, *Streptococcus* faecal count, faecal coliform and *Staphylococcus aureus* in minced beef meat collected from supermarkets at day 0 were 4.3, 3.2, 2.5, 2.2 and 3.9 log₁₀ CFU/g, respectively. These counts increased after 5 days of storage at 4±1 °C to 7.3, 7.1, 3.8, 5.0 and 3.3 log₁₀ CFU/g, respectively. These counts decreased after 6 months at -10±1 °C to 3.2, 2.6, 2.0, 1.2 and 1.0 log₁₀ CFU/g, respectively. The results also indicated that the total viable count of *Pseudomonas*, *Streptococcus* faecal count, faecal coliform and *Staphylococcus aureus* were higher in small butcher shop as compared to supermarket at day 0. On day 0 the thiobarbituric acid reactive in minced beef meat samples collected from supermarket and small butcher shop were 0.89 and 1.15 mg malonaldehyde/kg, respectively. After 5 days of storage at 4 °C, the thiobarbituric acid reactive in minced meat beef collected from supermarket and small shop increased and reached upto 2.95 and 3.74 mg malonaldehyde/kg, respectively. It increased to 3.02 in minced beef from supermarket, and 3.85 mg from small shop after 6 months at -10±1 °C. Lightness, redness and yellowness of minced beef meat decreased, when meat was kept under cooling and freezing temperature, however, lightness, redness and yellowness of minced beef meat were higher in density in supermarket samples than those of meat obtained from small butcher shops.

Keywords: cooling, freezing temperature, minced beef meat, TBARS, colour

Comparison of Antibacterial Activity of Crude Alkaloid and Saponin Extract from *Phyllanthus niruri*

Victor Adeyinka Ajibade

Department of Science Technology, Microbiology Option, Federal Polytechnic,
P. M. B. 5351, Ado-Ekiti, Ekiti State, Nigeria

(received February 26, 2013; revised July 31, 2013; accepted August 5, 2013)

Abstract. The antibacterial activity of crude extract of saponin and alkaloid from *Phyllanthus niruri* was investigated and compared against some test bacteria. With the activity of saponins, *Bacillus subtilis*, *Salmonella typhi* and *Klebsiella pneumoniae* were resistant at concentrations of 0.01 mg/mL and 0.02 mg/mL, while, they were susceptible at 0.03 mg/mL, 0.4 mg/mL and 0.05 mg/mL concentrations. With the activity of saponin and alkaloid, *B. subtilis*, *K. pneumoniae* and *S. typhi* were resistant at 0.01 mg/mL concentration, while, *Staphylococcus aureus* was resistant to alkaloid at 0.01 mg/mL concentration but susceptible to saponin at the same concentration. *Escherichia coli*, *Pseudomonas aeruginosa* and *S. aureus* were more sensitive to saponin with diameter of zone of inhibition of 8.00 mm, 12.00 mm and 12.00 mm compared with 5.00 mm, 10.00 mm and 10.00 mm, respectively, as observed in alkaloid. The test organisms were susceptible to saponin at a concentration of 0.03 mg/mL. *S. aureus* was resistant to alkaloid; *B. subtilis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi* and *S. aureus* were sensitive to both saponin and alkaloid. Combined, saponin and alkaloid showed more potency and may offer an alternative therapeutic agent against bacterial infections.

Keywords: antibacterial activity, alkaloid, *Phyllanthus niruri*, saponin

Utilization of Poultry Excreta for High Density Production of *Daphnia carinata* (King 1853): Cost Effective and Environmental Friendly Technique

Mehrajuddin War* and Kareem Altaff

Unit of Reproductive Biology and Live Feed Culture, Department of Zoology,
The New College Chennai - 600 014, India

(received October 2, 2012; revised July 8, 2013; accepted July 10, 2013)

Abstract. *Daphnia carinata* was cultured for 21 days using poultry excreta to fertilise the medium at the rate of 500 ppt and maximum density of 5633.32 ± 88 Ind./L was recorded on 11th day of culture in the tanks, where, the feed was administered with 25% dosage followed by 50% dosage (1894.44 ± 9.68 Ind./L) and 75% dosage (1103.55 ± 17.80 Ind./L) of the initial dosage (500 ppt). A 50% renewal of the medium thrice a week proved optimal for the population development. The water analysis showed that the temperature range of 28 ± 2 °C and pH of 6-7 was conducive for optimal growth of *D. carinata*.

Keywords. *Daphnia carinata*, chicken manure, live feed, fish farming, aquaculture

Short Communication

Heat Processing and Cold Storage Effects on Vitamins B₁ and B₂ of Buffalo Milk

Alim-un-Nisa*, Abdul Majeed Sularya, Sajila Hina and Shahid Masood

Food and Biotechnology Research Centre, PCSIR Laboratories Complex,
Ferozpur Road, Lahore-54600, Pakistan

(received May 28, 2012; revised May 2, 2013; accepted June 17, 2013)

Abstract. Heat processing and cold storage effects on vitamins B₁ and B₂ contents of whole and skimmed buffalo milk were investigated. Whole and skimmed buffalo milk was heated at various temperatures (90-140 °C) for different time periods (2-90 min). Losses in vitamins B₁ and B₂ occurred to various extents depending upon the temperature and time period of heating and the storage conditions. Maximum losses in vitamins B₁ and B₂ were found on heating milk at 110 °C for 90 min and 140 °C for 8 min. Maximum losses in vitamins B₁ and B₂ were found to be 32.5 and 29.9% at 110 °C and 37.5 and 32.6% at 140 °C for whole buffalo milk, 30.4 and 26.4% at 110 °C and 34.8 and 29.6% at 140 °C for skimmed buffalo milk, respectively. Similarly, after 15 days cold storage, maximum amount of vitamins B₁ and B₂ was lost from heated whole and skimmed buffalo milk. Losses in these two water soluble vitamins were comparatively higher in case of whole buffalo milk than skimmed buffalo milk after heat treatment. However, losses in vitamin B₁ were higher than vitamin B₂ contents in all samples.

Keywords: buffalo milk, vitamins B₁ and B₂, heat processing, cold storage

Short Communication

Biochemical Composition of Koi (*Anabas testudineus*) Collected from Paddy Field of Mymensingh, Bangladesh

Mohajira Begum^{a*} and Maruf Hossain Minar^b

^aFish Technology Research Section, Institute of Food Science and Technology,
Bangladesh Council of Scientific and Industrial Research, Dhanmondi, Bangladesh

^bDepartment of Fisheries Biology and Genetics, Bangladesh Agricultural University,
Mymensingh-2202, Bangladesh

(received February 12, 2013; revised June 19, 2013; accepted July 26, 2013)

Abstract. An experiment was undertaken to analyse proximate composition of Koi (*Anabas testudineus*), collected from a rice field from Mymensingh, Bangladesh. Fishes were grown as a second crop along with rice. Moisture, protein, lipid and ash percentage (%) were evaluated as 70.07 ± 1.10 , 16.97 ± 0.82 , 13.01 ± 0.47 , 0.95 ± 12 , respectively, in wet basis. When compared to previous reports, a lot of variation was observed in the case of lipid.

Keywords: proximate composition, *Anabas testudineus*, paddy field, lipid

Short Communication

First Record of *Cyphonotus testaceus* (Pallas, 1781) (Coleoptera: Scarabaeidae: Melolonthinae: Melolonthini) from Pakistan

Zubair Ahmed^{a*}, Aleš Bezděk^b and Muhammad Atique Akhter^c

^aDepartment of Zoology, Federal Urdu University of Arts, Science & Technology, Karachi, Pakistan

^bBiology Centre ASCR, Institute of Entomology, Branišovská 31, CZ-370 05
České Budějovice, Czech Republic

^cDepartment of Zoology, University of Karachi, Karachi, Pakistan

(received July 11, 2013; revised August 24, 2013; accepted August 29, 2013)

Abstract. The melolonthine species *Cyphonotus testaceus* (Pallas, 1781) is for the first time recorded from Pakistan. Geographic distribution of this rarely collected species is summarized. Habitus photo of the male collected in Pakistan is presented.

Keywords: *Cyphonotus testaceus*, new record, melolonthini, palaearctic region, oriental region, Pakistan
