PROCEEDINGS

OF

PAKISTAN CONGRESS OF ZOOLOGY

Volume 41 (2023)

PROCEEDINGS

OF

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Volume 41, 2023

All research articles in this Proceedings were refereed
by experts in respective disciplines

FORTY FIRST PAKISTAN CONGRESS OF ZOOLOGY
March 16-18, 2023

held under the auspices of

THE ZOOLOGICAL SOCIETY OF PAKISTAN

at

INSTITUTE OF ZOOLOGY,
UNIVERSITY OF THE PUNJAB, LAHORE

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PROCEEDINGS OF THE CONGRESS

Editor

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Composed & designed by:akhar Mahmood Shahid

ACKNOWLEDGMENTS

PROGRAMME

41ˢᵗ

PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)

March 16-18, 2023

Organized by:
**INSTITUTE OF ZOOLOGY
UNIVERSITY OF THE PUNJAB,
LAHORE**

41st PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)
UNIVERSITY OF THE PUNJAB
March 16 - March 18, 2023

DAY ONE   **THURSDAY, MARCH 16, 2023**

08:30 AM   **REGISTRATION**

09:00 AM   **Inauguration: Recitation from the Holy Quran**

09:05 AM   **Welcome address by Dean Faculty of Life Sciences**

09:10 AM   **Address by the Secretory General, Zoological Society of Pakistan**

09:20 AM   **Address by the President, Zoological Society of Pakistan**

09:30 AM   **Address by the Vice-chancellor, University of the Punjab, Lahore**

09:40 AM   **Distribution of Medals and Awards**

10:10 AM   **Address by the Chief Guest, Prof. Dr. Shahid Munir, Chairman, Punjab**

Higher Education Commission, Lahore.

10:35 AM   **Vote of Thanks**

10:40 AM   **Refreshment**

11:30 AM-JOINT SESSION I: PLENARY LECTURES
CHAIRPERSON: Prof Dr. Muhammad Arslan
CO-CHAIRPERSON: Prof. Dr. Javed Iqbal Qazi

1. **Prof. Dr. Andre J. Van Wijnen**, Department of Biochemistry, University of Vermont, Burlington, Vermont, USA
2. **Prof. Dr. Ma Tonghui, Nanjing** University of Chinese Medicine, Nanjing, China
   Research Progress of Liver Aquaporins.
3. **Prof. Dr. Javed Iqbal Qazi**, Institute of Zoology, University of the Punjab, Lahore
   Scientifically Strengthening The Dwindling Traditional Medicinal Practices and Local Wisdoms
4. **Prof. Dr. Diyan Li**, School of Pharmacy, , Chengdu University, Chengdu, Sichuan, China
   Vaginal microbes and female infertility

01:30 PM LUNCH AND PRAYER BREAK (ZUHAR)

02:15 PM - HALL-1		02:15 PM - HALL-2		02:15 PM - HALL-3	
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY SESSION 1 CHAIRPERSON: Prof. Dr. Farah Rauf Shakoori CO-CHAIRPERSON: Dr. Shagufta Naz		**SECTION III** ENTOMOLOGY SESSION 1 CHAIRPERSON: Dr. Zainul Abdin CO-CHAIRPERSON: Dr. Santosh Kumar		**SECTION V** FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY SESSION 1 CHAIRPERSON: Prof. Dr. Zulfikar Ali CO-CHAIRPERSON: Dr. Sangam Khalil	
1. I. Zahra (UP, Lahore)	CBGP-1	1. S. Mangi (SALU, Khairpur)	ENT-1	1. N. Bano (MNSUA, Multan)	FEWFM-1
2. A. Nisar (FBRC)	CBGP-2	2. S.t. Muntha (US, Jamshoro)	ENT-2	2. S. Lakhair (US, Jamshoro)	FEWFM-2
3. A. Raza (GDEC)	CBGP-3	3. M. Nawab (US, Jamshoro)	ENT-3	3. S. Sheharyar (US, Jamshoro)	FEWFM-3
4. S.Y. Khan (US, Sargodha)	CBGP-4	4. I.A. Soomro (US, Jamshoro)	ENT-4	4. B.A. Dars (US, Jamshoro)	FEWFM-4
5. M. Rasheed (UK, Karachi)	CBGP-5	5. V. Kumari (US, Jamshoro)	ENT-5	5.	
6. S. Naz (UK, Karachi)	CBGP-6	6. S. Sanam (CABI, R'pindi)	ENT-6	6. N.T. Narejo (US, Jamshoro)	FEWFM-5
7. S. Ahmad (BZU, Multan)	CBGP-7	7. M.S. Dayo (US, Jamshoro)	ENT-7	7. M. Rafiq (UoL, Lahore)	FEWFM-6
8. N. Ghafoor (UA, Faisalabad)	CBGP-8	8. S.A. Talpur (US, Jamshoro)	ENT-8	8. K. Tayyab (UoL, Lahore)	FEWFM-7
9. M. Iqbal (GCU, Lahore)	CBGP-9	9. S.P. Memon (US, Jamshoro)	ENT-9	9. A. Saleem (UoL, Lahore)	FEWFM-8
10. A. Akram (FUUAST, Karachi)	CBGP-10	10. S. Soomro (US, Jamshoro)	ENT-10	10. A. Majid (UoL, Lahore)	FEWFM-9
11. G. Mustafa (GCU, Lahore)	CBGP-11	11. S. Sanam (US, Jamshoro)	ENT-11	11. S. Siddique (LUAWMS, Uthal)	FEWFM-10
12. Noor-ul-Huda (GCU, Lahore)	CBGP-12	12. R. Shah (US, Jamshoro)	ENT-12	12. A. Ashraf (UA, Faisalabad)	FEWFM-11
13. M. Haider (UP, Lahore)	CBGP-13	13. N. Soomro (US, Jamshoro)	ENT-13	13. A. Karim (SBKWU, Quetta)	FEWFM-12
14. M. Rani (UP, Lahore)	CBGP-14	14. N.G. Khattak (US, Jamshoro)	ENT-14	14. A. Qadir (UP, Lahore)	FEWFM-13
15. A. Arshad (UP, Lahore)	CBGP-15	15. N. Bhanger (US, Jamshoro)	ENT-15	15. S. Riaz (UK, Karachi)	FEWFM-14
				16. D.S. Thebo (US, Jamshoro)	FEWFM-15
				17. M.F. Ali (UP, Peshawar)	FEWFM-16
				18. S. Nasreen (UK, Karachi)	FEWFM-17

04:30 PM TEA BREAK AND PRAYER BREAK (ASAR)

05:00 PM - HALL-1 — SECTION I
CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY
SESSION 2
CHAIRPERSON: Prof. Dr. Abdul Rehman
CO-CHAIRPERSON: DR. Chaman Ara

#	Presenter	Code
1.	H. Amjad (LCWU, Lahore)	CBGP-16
2.	A. Anwar (UP, Lahore)	CBGP-17
3.	A. Sania (UP, Lahore)	CBGP-18
4.	U. Momal (MNSUA, Multan)	CBGP-19
5.	Q. Qadeer (KCWU, Lahore)	CBGP-20
6.	A. Hussain (UK, AJK)	CBGP-21
7.	A.T.A. Khan (UP, Lahore)	CBGP-22
8.	Q. Fatima (UP, Lahore)	CBGP-23
9.	A. Khawer (UoL, Lahore)	CBGP-24
10.	S. Naz (LCWU, Lahore)	CBGP-25
11.	Ayesha (UP, Peshawar)	CBGP-26
12.	A. Fargoson (US, Sialkot)	CBGP-27
13.	T. Ahmed (MUST, Mirpur, AJK)	CBGP-28
14.	S. Khushal (MUST, Mirpur)	CBGP-29
15.	A.A. Abbasi (MUST, Mirpur)	CBGP-30
16.	S. Roshan (UG, Gujrat)	CBGP-31

05:00 PM - HALL-2 — SECTION III
ENTOMOLOGY
SESSION 2
CHAIRPERSON: Prof. Dr. Shafqat Saeed
CO-CHAIRPERSON: Dr. Waheed Ali Panhwar

#	Presenter	Code
1.	M.A. Jakhrani (US, Jamshoro)	ENT-16
2.	M. Ali (US, Jamshoro)	ENT-17
3.	M. Jamil (US, Jamshoro)	ENT-18
4.	M.I. Bozdar (US, Jamshoro)	ENT-19
5.	F.D. Soomro (US, Jamshoro)	ENT-20
6.	B.A. Bughio (US, Jamshoro)	ENT-21
7.	A. Soomro (US, Jamshoro)	ENT-22
8.	A. Aziz (US, Jamshoro)	ENT-23
9.	A. Chandio (US, Jamshoro)	ENT-24
10.	A. Akhtar (US, Jamshoro)	ENT-25
11.	A.N. Memon (GUBDC, Dadu)	ENT-26
12.	A.R. Soomro (US, Jamshoro)	ENT-27
13.	A. Iqbal (CUVAS, Bahawalpur)	ENT-28
14.	S. Tariq (CUVAS, Bahawalpur)	ENT-29
15.	M.Z.U. Abedin (CUVAS, Bahawalpur)	ENT-30

05:00 PM - HALL-3 — SECTION V
FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY
SESSION 2
CHAIRPERSON: Prof. Dr. Noor Khan
CO-CHAIRPERSON: Dr. Muhammad Rais

#	Presenter	Code
1.	F. Asad (GCU, Faisalabad)	FEWFM-18
2.	M. Mahwish (JUW, Karachi)	FEWFM-19
3.	T. Baby (JUW, Karachi)	FEWFM-20
4.	R. Hadi (JUW, Karachi)	FEWFM-21
5.	Z. Iqbal (UP, Lahore)	FEWFM-22
6.	Habibullah (LUAWMS, Uthal)	FEWFM-23
7.	N. Shoaib (UK, Karachi)	FEWFM-24
8.	R. Yasmeen (UK, Karachi)	FEWFM-25
9.	S. Khanam (UK, Karachi)	FEWFM-26
10.	Q.B. Kazmi (UK, Karachi)	FEWFM-27
11.	R. Bibi (UK, Karachi)	FEWFM-28
12.	S. Bibi (UK, Karachi)	FEWFM-29
13.	S. Rasheed (LUAWMS, Uthal)	FEWFM-30
14.	S. Bano (UK, Karachi)	FEWFM-31
15.	F.M. Shoab (UK, Karachi)	FEWFM-32
16.	T. Hamid (UK, Karachi)	FEWFM-33
17.	M.A. Mahar (US, Campus Thatta)	FEWFM-34

6:25 PM PRAYER BREAK (MAGHRB)

06:40 PM - HALL-1	06:40 PM - HALL-2	06:40 PM - HALL-3
SECTION I	SECTION III	SECTION V
CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY	ENTOMOLOGY	FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY
SESSION 3	SESSION 3	SESSION 3
CHAIRPERSON: Dr. M. Afzal Ghauri	CHAIRPERSON: Mr. Abdul Aziz Khan	CHAIRPERSON: Prof. Dr. Naeem Tariq Narejo
CO-CHAIRPERSON: Dr. Bushra Muneer	CO-CHAIRPERSON: Prof. Dr. Asmatullah Kakar	CO-CHAIRPERSON: Dr. Noor us Saher
1. A. Ali (US, Sargodha) — CBGP-32	1. M. Irfan (CUVAS, Bahawalpur) — ENT-31	1. A. Rauf (UAJK, Muzaffarabad) — FEWFM-35
2. Z. Yousaf (UP, Lahore) — CBGP-33	2. M. Nazir (CUVAS, Bahawalpur) — ENT-32	2. A. Abbas (UK, Karachi) — FEWFM-36
3. A. Zaman (UP, Lahore) — CBGP-34	3. M. Waseem (CUVAS, Bahawalpur) — ENT-33	3. A. Jan (NIOR, Gwadar) — FEWFM-37
4. S. Batool (LGU, Lahore) — CBGP-35	4. H.M. Shahzad (CUVAS, Bahawalpur) — ENT-34	4. M. Tabish (UK, Karachi) — FEWFM-38
5. S. Odhano (SBBUVAS, Sakrand) — CBGP-36	5. Z. Iqbal (CUVAS, Bahawalpur) — ENT-35	5. A. Hameed (UK, Karachi) — FEWFM-39
6. A. Khalid (LCWU, Lahore) — CBGP-37	6. K. Ahmad (CUVAS, Bahawalpur) — ENT-36	6. N. Hawa (UK, Karachi) — FEWFM-40
7. A. Riaz (LCWU, Lahore) — CBGP-38	7. L. Zahra (CUVAS, Bahawalpur) — ENT-37	7. A.H. Narejo (UK, Karachi) — FEWFM-41
8. F. Naz (UK, Karachi) — CBGP-39	8. M. Luqman (US, Jamshoro) — ENT-38	8. D. Shahwar (UK, Karachi) — FEWFM-42
9. T. Ali (GCU, Faisalabad) — CBGP-40	9. R.Z. Khizar (MNUA, Multan) — ENT-39	9. S.H. Noor (UK, Karachi) — FEWFM-43
10. H.A. Sajjad (MNSUA, Multan) — CBGP-41	10. F. Hussain (MNUA, Multan) — ENT-40	10. N.U. Saher (UK, Karachi) — FEWFM-44
11. F. Anwaar (UVAS, Lahore) — CBGP-42	11. Z.U.A. Abro (US, Jamshoro) — ENT-41	11. W.M. Zaheen (UK, Karachi) — FEWFM-45
12. S.F.A. Jafferi (UK, Karachi) — CBGP-43	12. P.A. Ali (WU, Swabi) — ENT-42	12. R. Tahir (UK, Karachi) — FEWFM-46
13. N. Fatima (GCU, Lahore) — CBGP-44	13. H. Lakho (US, Jamshoro) — ENT-43	13. M.U. Ain (UK, Karachi) — FEWFM-47
14. M.Q. Sarwar (GCU, Lahore) — CBGP-45	14. H. Saand (US, Jamshoro) — ENT-44	14. A. Khan (BZU, Multan) — FEWFM-48
15. M. Idrees (GCU, Lahore) — CBGP-46	15. N. Bloch (US, Jamshoro) — ENT-45	15. S. Aslam (UP, Lahore) — FEWFM-49
16. S. Ali (GCU, Lahore) — CBGP-47		16. K. Mahmood (UP, Lahore) — FEWFM-50
		17. A. Ghaus (UP, Lahore) — FEWFM-51

EXECUTIVE COUNCIL MEETING

8:30 PM DINNER

DAY TWO: FRIDAY, MARCH 17, 2023

09:00 AM-JOINT SESSION II: PLENARY LECTURES
CHAIRPERSON: Prof. Dr. Muhammad Afzal
CO-CHAIRPERSON: Dr. Abdul Aleem Chaudhary

1. **Prof. Dr. Muhammad Munir**, Division of Biomedical and Life Sciences, Lancaster University, Lancaster, UK
   Zoonotic And Zooanthroponotic Transmission Of Sars-Cov-2
2. **Prof. Dr. Naveeda Akhtar Qureshi**, Department of Animal Sciences, Quaid-i-Azam University, Islamabad
   Prunus armeniaca A Potential Agent for Drug Formulation Against Cutaneous Leishmaniasis: An Emerging Tropical Disease in Pakistan
3. **Dr. Muhammad Akhtar Ali**, School of Biological Sciences, University of the Punjab, Lahore
   Functional Genetic and Computational Approaches for Complex Phenotypes and Drug Screens
4. **Prof. Dr. Mohammad Perwaiz Iqbal**, Department of Life Sciences, University of Management and Technology, Lahore
   Air Pollution: Effect of Toxic Elements on Health in Pakistan

10:30 AM - HALL-1		10:30 AM - HALL-2		10:30 AM - HALL-3	
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY SESSION 4 CHAIRPERSON: Dr. Muhammad Khan CO-CHAIRPERSON: Dr. Soumble Zulfiqar		**SECTION III** ENTOMOLOGY SESSION 4 CHAIRPERSON: Prof. Dr. Abida Butt CO-CHAIRPERSON: Prof. Dr. Hafiz M. Tahir		**SECTION V** FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY SESSION 4 CHAIRPERSON: Prof. Dr. Muhammad Akbar Khan CO-CHAIRPERSON: Dr. Sadaf Aslam	
1. M. Ahmad (GCU, Lahore)	CBGP-48	1. S. Kaka (US, Jamshoro)	ENT-46	1. M.K. Nawaz (UP, Lahore)	FEWFM-52
2. Z. Bibi (GCU, Lahore)	CBGP-49	2. M.Z. Ali (MNUA, Multan)	ENT-47	2. T. Shahzad (UP, Lahore)	FEWFM-53
3. B. Gulzar (GCU, Lahore)	CBGP-50	3. R. Khan (MNUA, Multan)	ENT-48	3. K. Aftab (UG, Gujrat)	FEWFM-54
4. Areeba (GCU, Lahore)	CBGP-51	4. Z. Rajput (SAU, Tandojam)	ENT-49	4. F. Jaber (UO, Okara)	FEWFM-55
5. A. Sarwar (UK, Karachi)	CBGP-52	5. H. Mannan (SAU, Tandojam)	ENT-50	5. M.A. Babar (UO, Okara)	FEWFM-56
6. M.N. Awan (GCU, Lahore)	CBGP-53	6. A.P. Soomro (SAU, Tandojam)	ENT-51	6. Q. Tasneem (MU, Lahore)	FEWFM-57
7. S. Iqbal (UK, Karachi)	CBGP-54	7. M.U. Brohi (SAU, Tandojam)	ENT-52	7. H. Ayuab (IUB, Bahawalpur)	FEWFM-58
8. K. Malik (UP, Lahore)	CBGP-55	8. S.B. Mustafa (SALU, Khairpur)	ENT-53	8. H. Habib (IUB, Bahawalpur)	FEWFM-59
9. S. Younas (GCU, Lahore)	CBGP-56	9. S.R. Soomro (SALU, Khairpur)	ENT-54	9. U. Majeed (IUB, Bahawalpur)	FEWFM-60
10. A. Hasan (UP, Lahore)	CBGP-57	10. Roomasa (SALU, Khairpur)	ENT-55	10. S. Batool (IUB, Bahawalpur)	FEWFM-61
11. F. Ghazanfar (UVAS, Lahore)	CBGP-58	11. W.A. Panhwar (SALU, Khairpur)	ENT-56	11. S.U. Rehman (UB, Quetta)	FEWFM-62
12. I. Liaqat (GCU, Lahore)	CBGP-59	12. S.A. Larik (SALU, Khairpur)	ENT-57	12. T. Mahmood (PMASAAU, Rawalpindi)	FEWFM-63
13. N. Muhammad (GCU, Lahore)	CBGP-60	13. B.A. Samejo (SALU, Khairpur)	ENT-58	13. G. Zaman (UVAS, Lahore)	FEWFM-64
14. A. Rehman (UP, Lahore)	CBGP-61	14. A. Mangi (SALU, Khairpur)	ENT-59	14. R. Ahmad (LCWU, Lahore)	FEWFM-65
15. M.T. Zahid (GCU, Lahore)	CBGP-62	15. A.A. Mirbahar (SALU, Khairpur)	ENT-60	15. H. Waseem (UP, Lahore)	FEWFM-66
16. S. Mushtaq (UP, Lahore)	CBGP-63			16. R. Riaz (UP, Lahore)	FEWFM-67
				17. M.N. Faisal (UP, Lahore)	FEWFM-68

11:45 AM - TEA BREAK

12:00 NOON - HALL-1	12:00 NOON - HALL-2	12:00 NOON - HALL-3
SECTION 1	**SECTION III**	**SECTION V**
CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY	ENTOMOLOGY	FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY
SESSION 5	SESSION 5	SESSION 5
CHAIRPERSON: Prof. Dr. Rubina Mushtaq	CHAIRPERSON: Dr. Riffat Sultana	CHAIRPERSON: Dr. Zafar Iqbal
CO-CHAIRPERSON: Dr. Dil Ara Abbas Bukhari	CO-CHAIRPERSON: Dr. Abro Zain	CO-CHAIRPERSON: Dr. Abdullah Shakir
1. I. Shahid (UP, Lahore) — CBGP-64	1. G.Z. Khan (NIFA, Peshawar) — ENT-61	1. F. Anjum (UP, Lahore) — FEWFM-69
2. M. Ashraf (KCWU, Lahore) — CBGP-65	2. I. Usman (AWKU, Multan) — ENT-62	2. B. Mustafa (GCU, Lahore) — FEWFM-70
3. H. Saeed (UP, Lahore) — CBGP-66	3. M.J. Iqbal (IUB, Bahawalpur) — ENT-63	3. A. Abbas (GCU, Lahore) — FEWFM-71
4. A. Haq (UP, Lahore) — CBGP-67	4. A. Rehman (MNUA, Multan) — ENT-64	4. M. Irfan (UoL, Lahore) — FEWFM-72
5. S. Saleemi (UP, Lahore) — CBGP-68	5. S.H.M. Bokhari (MNUA, Multan) — ENT-65	5. F. Chaudhary (UVAS, Lahore) — FEWFM-73
6. M.F. Maqbool (UP, Lahore) — CBGP-69	6. Z. Bano (KUST, Kohat) — ENT-66	6. F. Ameer (BZU, Multan) — FEWFM-74
7. I. Zafar (UP, Lahore) — CBGP-70	7. A. Hameed (MRI, Multan) — ENT-67	7. S. Riaz (UP, Rawalakot) — FEWFM-75
8. S. Rizwana (UP, Lahore) — CBGP-71	8. A.H. Khan (MRI, Multan) — ENT-68	8. M.R. Azam (UP, Rawalakot) — FEWFM-76
9. F. Ambreen (GCWU, Fisalabad) — CBGP-72	9. S. Ahmad (UA, Faisalabad) — ENT-69	9. A. Ashraf (UP, Rawalakot) — FEWFM-77
10. R. Shaukat (MUST, Mirpur) — CBGP-73	10. M.A. Shah (US, Jamshoro) — ENT-70	10. F. Bari (UC, Chitral) — FEWFM-78
11. F. Nazir (CUST, Islamabad) — CBGP-74	11. N.A. Shah (US, Jamshoro) — ENT-71	11. S. Hamid (UVAS, Lahore) — FEWFM-79
12. M.A. Prince (US, Jamshoro) — CBGP-75	12. M. Tayyib (UA, Faisalabad) — ENT-72	12. M. Iqbal (UAJK, Muzaffarabad) — FEWFM-80
13. A.W. Qureshi (GCWU, Sialkot) — CBGP-76	13. K. Saeed (UB, Buner) — ENT-73	13. A. Kazmi (UAJK, Muzaffarabad) — FEWFM-81
14. K.A. Akbar (UA, Fisalabad) — CBGP-77		14. S. Abbasi (UAJK, Muzaffarabad) — FEWFM-82
15. A. Sadaf (UA, Fisalabad) — CBGP-78		15. B. Ahmad (UAJK, Muzaffarabad) — FEWFM-83
16. M.T. Munawar (UA, Fisalabad) — CBGP-79		16. V. Mubeen (UVAS, Lahore) — FEWFM-84
		17. U. Ali (MUST, Mirpur) — FEWFM-85

01:30 PM LUNCH BREAK (ZUHAR)

x

02:00 PM - HALL-1	02:00 PM - HALL-2	02:00PM - HALL-3
SECTION 1	**SECTION II**	**SECTION V**
CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY	PESTS AND PEST CONTROL	FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY
SESSION 6	SESSION 1	SESSION 6
CHAIRPERSON: Prof. Dr. Saima Sharif	CHAIRPERSON: Prof. Dr. Safia Khanum	CHAIRPERSON: Dr. Azra Bano
CO-CHAIRPERSON: Dr. Ali Hussain	CO-CHAIRPERSON: Dr. Ayesha Aihetasham	CO-CHAIRPERSON: Dr. Iftikhar Ahmad
1. J. Taj (US, Sialkot) — CBGP-80	1. A.A. Samejo (GBDC, Umerkot) — PC-1	1. I. Aslam (LGU, Lahore) — FEWFM-86
2. S. Sitara (US, Sialkot) — CBGP-81	2. S. Laraib (US, Jamshoro) — PC-2	2. T. Sadaqat (PMASAAU, Rawalpindi) — FEWFM-87
3. M. Maqsood (LCWU, Lahore) — CBGP-82	3. S.A. Mahar (US, Jamshoro) — PC-3	3. I. Aziz (Vu, Lahore) — FEWFM-88
4. A. Azam (SBBWU, Peshawar) — CBGP-83	4. S. Soomro (US, Jamshoro) — PC-4	4. K. Ahmed (UP, Lahore) — FEWFM-89
5. M.J. Khilji (US, Jamshoro) — CBGP-84	5. M. Noman (US, Jamshoro) — PC-5	5. H. Lakho (US, Jamshoro) — FEWFM-90
6. N.Z. Khan (US, Jamshoro) — CBGP-85	6. M.M. Jakhrani (US, Jamshoro) — PC-6	6. G.M. Moroojo (US, Jamshoro) — FEWFM-91
7. A. Khan (UK, Karachi) — CBGP-86	7. M. Ali (US, Jamshoro) — PC-7	7. I.R. Shaikh (US, Jamshoro) — FEWFM-92
8. N.M. Ghanim (UK, Karachi) — CBGP-87	8. M.M. Hussain (US, Jamshoro) — PC-8	8. G.S. Gachal (US, Jamshoro) — FEWFM-93
9. M. Kamal (AWKU, Mardan) — CBGP-88	9. K. Bai (US, Jamshoro) — PC-9	9. M. Shafqat (QU, Islamabad) — FEWFM-94
10. A. Parveen (GDEC, Lahore) — CBGP-89	10. J. Das (US, Jamshoro) — PC-10	10. A.R. Shaikh (US, Jamshoro) — FEWFM-95
11. S. Khan (US, Swabi) — CBGP-90	11. F.A. Hussain (US, Jamshoro) — PC-11	11. M. Tahir (UA, Faisalabad) — FEWFM-96
12. S. Kousar (GCWU, Faisalabad) — CBGP-91	12. F. Anum (US, Jamshoro) — PC-12	12. W. Mustfa (UA, Faisalabad) — FEWFM-97
13. A. Malik (BZU, Multan) — CBGP-92	13. F. Abbasi (US, Jamshoro) — PC-13	13. S. Mirza (UK, Karachi) — FEWFM-98
14. S.U. Bukhari (UP, Lahore) — CBGP-93	14. A. Bari (US, Jamshoro) — PC-14	14. R.M. Tariq (UK, Karachi) — FEWFM-99
15. K. Nisa (UG, Gujrat) — CBGP-94	15. Ayesha (US, Jamshoro) — PC-15	15. S. Irum (UG, Gujrat) — FEWFM-100
16. T. Fatima (UA, Fisalabad) — CBGP-95		16. A. Sajjad (UP, Lahore) — FEWFM-101

04:30 PM TEA BREAK AND PRAYER BREAK (ASAR)

05:00 PM - HALL-1		05:00 PM - HALL-2		05:00PM - HALL-3	
SECTION 1 **CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY** **SESSION 7** CHAIRPERSON: Prof. Dr. Nadeem Shaikh CO-CHAIRPERSON: Dr. Iram Liaqat		**SECTION II** **PESTS AND PEST CONTROL** **SESSION 2** CHAIRPERSON: Prof. Dr. Amjad Farooq CO-CHAIRPERSON: Prof. Dr. Imran Khatri		**SECTION V** **FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY** **SESSION 7** CHAIRPERSON: Prof Dr. Abdul Majid Khan CO-CHAIRPERSON: Prof. Dr. Abdullah G. Arijo	
1.	M. Khalil (UA, Faisalabad)	CBGP-96	17.	A.J. Hussain (US, Jamshoro)	PC-16	31.	A. Rahim (PMASAAU, Rawalpindi)	FEWFM-102
2.	Z. Latif (UAJK, Muzaffarabad)	CBGP-97	18.	F. Farah (JUW, Karachi)	PC-17	32.	A.U. Rehman (UP, Lahore)	FEWFM-103
3.	G.M. Ghani (PHC, DG Khan)	CBGP-98	19.	H. Sultan (JUW, Karachi)	PC-18	33.	Z. B. Mirza (IUCN Pakistan)	FEWFM-104
4.	A. Shah (US, Jamshoro)	CBGP-99	20.	A. Kakar (UB, Quetta)	PC-19	34.	U. Ahmad (UP, Lahore)	FEWFM-105
5.	R. Mushtaq (FUUAST, Karachi)	CBGP-100	21.	S. Memon (SALU, Khairpur)	PC-20	35.	A. Naeem (GCS, Lahore)	FEWFM-106
6.	S. Khwaja (FUUAST, Karachi)	CBGP-101	22.	Z. Hussain (BZU, Multan)	PC-21	36.	M. Mustafa (UAJK, Muzaffarabad)	FEWFM-107
7.	Inamullah (AWKU, Mardan)	CBGP-102	23.	A. Amur (US, Jamshoro)	PC-22	37.	F. Naaz (UP, Lahore)	FEWFM-108
8.	A. Savanur (UK, Karachi)	CBGP-103	24.	M. Younus (CUVAS, Bahawalpur)	PC-23	38.	M.A. Haider (UP, Lahore)	FEWFM-109
9.	M. Riaz (WU, Swabi)	CBGP-104	25.	A. Siddiq (CUVAS, Bahawalpur)	PC-24	39.	M. Faizan (UP, Lahore)	FEWFM-110
10.	Z. Hassan (GCU, Lahore)	CBGP-105	26.	S. Kumar (CUVAS, Bahawalpur)	PC-25	40.	N. Kakar (UT, Turbat)	FEWFM-111
11.	Z. Zafar (107) (GCU, Lahore)	CBGP-106	27.	T. Kauser (GCWU, Siakot)	PC-26	41.	S. Amjad (IBM, Karachi)	FEWFM-112
12.	M. Rashid (GCU, Lahore)	CBGP-107	28.	N. Munir (LCWU, Lahore)	PC-27	42.	A. Saleem (LGU, Lahore)	FEWFM-113
13.	K. Shahzadi (GCU, Lahore)	CBGP-108	29.	M.H. Khan (NIFA, Peshawar)	PC-28	43.	H. Moin (JUW, Karachi)	FEWFM-114
14.	A. Haider (GCU, Lahore)	CBGP-109	30.	M. Zahid (NIFA, Peshawar)	PC-29	44.	M. Ahmed (SBB UVAS, Sakrand)	FEWFM-116
15.	F. Channa (US, Jamshoro)	CBGP-110						
16.	A. Basharat (GCU, Lahore)	CBGP-111						

08:00 PM GENERAL BODY MEETING

08:30 PM DINNER

DAY THREE: SATURDAY, MARCH 18, 2023

09:00 AM-JOINT SESSION III: PLENARY LECTURES
CHAIRPERSON: Prof. Dr. Mohummad Farooque Hassan
CO-CHAIRPERSON: Mr Abdul Aziz Khan

1. **Prof. Dr. Riffat Sultana**, Department of Zoology, University of Sindh, Jamshoro
Extinction Crisis of Orthoptera: Strategic Conservation Plan and Future Prospects

2. **Prof. Dr. Abida Butt**, Institute of Zoology, University of the Punjab, Lahore
Ecological Services Provided by Spiders in Agroecosystems of Pakistan

3. **Prof. Dr. Abdul Rauf Shakoori**, Cancer Research Center, University of the Punjab, Lahore; School of Biological Sciences, University of the Punjab, Lahore
Role of Slit2-ROBO1 and RhoA cAMP Signaling in Metastatic Phenotype

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10:30 AM - HALL-1
SECTION 1
CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY
SESSION 8
CHAIRPERSON: Prof. Dr. Nabila Roohi
CO-CHAIRPERSON: Dr. Muhammad Akhtar Ali

#	Presenter	Code
1.	N. Shah (US, Jamshoro)	CBGP-112
2.	R.M. Ahmad (GCU, Lahore)	CBGP-113
3.	H.M.S. Ali (UVAS, Lahore)	CBGP-114
4.	S. Shokat (GCU, Lahore)	CBGP-115
5.	T. Fatima (US, Sialkot)	CBGP-116
6.	F. Rasheed (US, Sialkot)	CBGP-117
7.	A. Shahzadi (QU, Islamabad)	CBGP-118
8.	M.S. Z. Khan (UP, Lahore)	CBGP-119
9.	H. Afaqi (QU, Islamabad)	CBGP-120
10.	I.Z. Qureshi (QU, Islamabad)	CBGP-121
11.	S. Khadam (QU, Islamabad)	CBGP-122
12.	A. Khan (QU, Islamabad)	CBGP-123
13.	A. Khalid (US, Sargodha)	CBGP-124
14.	S. Shahnawaz (WU, Multan)	CBGP-125
15.	S. Iqbal (WU, Multan)	CBGP-126
16.	S. Fatima (WU, Multan)	CBGP-127

10:30 AM - HALL-2
SECTION II
PESTS AND PEST CONTROL
SESSION3
CHAIRPERSON: Prof. Dr. Bushra Nisar Khan
CO-CHAIRPERSON: Dr. Inamullah Khan

#	Presenter	Code
1.	N. Yunus (UPR, Rawalakot)	PC-30
2.	K. Ali (AWKU, Mardan)	PC-31
3.	M. Baquar (SALU, Khairpur)	PC-32
4.	A. Marwat (GGC, DI Khan)	PC-33
5.	M.M.U. Haq (NIFA, Peshawar)	PC-34
6.	U. Khalique (NIFA, Peshawar)	PC-35
7.	K. Azam (MNSUA, Multan)	PC-36
8.	I Maryyam(MNSUA, Multan)	PC-37
9.	I. Khan (NIFA, Peshawar)	PC-38
10.	H. Faryal (MNSUA, Multan)	PC-39
11.	H. Ullah (MNSUA, Multan)	PC-40
12.	B.K. Solangi (SAU, Tandojam)	PC-41
13.	M.K. Mukhtar (US, Sargodha)	PC-42
14.	R. Ijaz (UP, Lahore)	PC-43

10:30 AM - HALL-3
SECTION IV
PARASITOLOGY
SESSION 1
CHAIRPERSON: Prof. Dr. Syed Akram Shah
CO-CHAIRPERSON: Dr. Nadir Ali Birmani

#	Presenter	Code
1.	I.A. Kapri (US, Jamshoro)	PAR-1
2.	I. Ahmad (SBBU, Sheringal)	PAR-2
3.	M. Rajper (US, Jamshoro)	PAR-3
4.	M. Nauman	PAR-4
5.	S.M.F. Shah (US, Jamshoro)	PAR-5
6.	T. Leghari (US, Jamshoro)	PAR-6
7.	S.N. Das (US, Jamshoro)	PAR-7
8.	Sajjad A. (UK, Karachi)	PAR-8
9.	W.S. Fatima (UK, Karachi)	PAR-9
10.	A.S. Hulio (US, Jamshoro)	PAR-10
11.	I. Mushtaq (UVAS, Lahore)	PAR-11
12.	I. Dad (UK, Karachi)	PAR-12
13.	A. Sattar (UK, Karachi)	PAR-13

11:45 AM - TEA BREAK

12:00 NOON - HALL-3

SECTION 1
CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY
SESSION 9
CHAIRPERSON: Dr. Mian Abdul Wajid
CO-CHAIRPERSON: Dr. Shafaat Yar Khan

#		
1.	S. Nawaz (UoL, Lahore)	CBGP-128
2.	S. Abbas (UJ, Jhang)	CBGP-129
3.	A. Marium (UVAS, Lahore)	CBGP-130
4.	A.A. Latif (LCWU, Lahore)	CBGP-131
5.	S. Rafiq (UA, Faisalabad)	CBGP-132
6.	M. Yaseen (UA, Faisalabad)	CBGP-133
7.	L. Majeed (UA, Faisalabad)	CBGP-134
8.	K. Alvi (UA, Fqisalabad)	CBGP-135
9.	A. Akhtar (UA, Faisalabad)	CBGP-136
10.	A. Ateeq (GCWU, Faisalabad)	CBGP-137
11.	I. Zulfiqar (UVAS, Lahore)	CBGP-138
12.	S. Tahreem (IUB, Bahawalpur)	CBGP-139
13.	M. Zahra (UP, Lahore)	CBGP-140
14.	S. Rasheed (UP, Lahore)	CBGP-141
15.	S. Rani (GCU, Lahore)	CBGP-142
16.	S. Naqvi (BZU, Multan)	CBGP-143

12:00 NOON - HALL-3

SECTION 1
CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY
SESSION 10
CHAIRPERSON: Prof. Dr. Ali Muhammad Yousafzai
CO-CHAIRPERSON: Prof. Dr. Asmatullah

#		
1.	A. Hayat (UA, Faisalabad)	CBGP-144
2.	N. Hussain (UP, Lahore)	CBGP-145
3.	M Arshad (UP, Lahore)	CBGP-146
4.	R. Azmat (UA, Faisalabad)	CBGP-147
5.	N. Ehsan (UA, Fqisalabad)	CBGP-148
6.	A. Khattak (UP, Peshawar)	CBGP-149
7.	M. Khalid (UAJK, Muzaffarabad)	CBGP-150
8.	Z.U. Rahman (UPR, Rawalakot)	CBGP-151
9.	Y. Samad (MNSUA, Multan)	CBGP-152
10.	M. Nazir (QU, Islamabad)	CBGP-153
11.	S. Hassan (QU, Islamabad)	CBGP-154
12.	H. Sadia (QU, Islamabad)	CBGP-155
13.	S.B. Shahid (QU, Islamabad)	CBGP-156
14.	M. Rizwan (GCU, Lahore)	CBGP-157
15.	F. Tabssum (EU, Lahore)	CBGP-158
16.	A. Liaqat (PU, Lahore)	CBGP-159
17.	S. Y. Khan (US, Sargodha)	CBGP-160

12:00 NOON - HALL-3

SECTION IV
PARASITOLOGY
SESSION 2
CHAIRPERSON: Dr. Aly Khan
CO-CHAIRPERSON: Dr. Wali Khan

#		
1.	T. Fatima (US, Jamshoro)	PAR-14
2.	B. Sadaf (US, Jamshoro)	PAR-15
3.	T. Mukhtar (PMASAAU, Rawalpindi)	PAR-16
4.	M. Saleem (SBBU, Sheringal)	PAR-17
5.	S. Naz (US, Jamshoro)	PAR-18
6.	F. Shaikh (US, Jamshoro)	PAR-19
7.	Jemi (US, Jamshoro)	PAR-20
8.	H. Lashari (US, Jamshoro)	PAR-21
9.	W. Hanif (GCS, Lahore)	PAR-22
10.	R. Malik (UK, Karachi)	PAR-23
11.	S.N. Panhwer (SAU, Sichuan, China)	PAR-24
12.	S. Hingoro (US, Jamshoro)	PAR-25
13.	K.T. Kubra (US, Jamshoro)	PAR-26

02:00 PM LUNCH BREAK (ZUHAR)

03:00 PM CONCLUDING CEREMONY

03:00 PM	Recitation
03:05 PM	Congress Report by Secretory ZSP
03:15 PM	Award Ceremony
03:45 PM	Concluding Remarks by the Chief Guest
03:55 PM	Vote of Thanks

04:00 PM REFRESHMENT

MEMBERS OF THE CONGRESS

BAHAWALPUR
Ayub, H.
Batool, S.
Habib, H.
Hassan, M.W. (Dr.)
Irfan, M.
Khalil, S. (Dr.)
Kumar, S. (Dr.)
Lashari, M.H. (Dr.)
Majeed, M.U.
Nazir, M.
Shahzad, H.M.
Siddiq, A.
Tahreem, S.
Tariq, S.
Waseem, M.
Younus, M.
Zain-ul-Abedin, M.

CHITRAL
Bari, F.

DERA ISMAIL KHAN
Ghani, G.M.A.

FAISALABAD
Azmat, R.
Ghafoor, N.
Hamza, A.
Hayat, M.F.
Nadeem, S. (Dr.)
Sattar, M.
Sheikh, Z.

GÖTTINGEN (Germany)
Saleem, T.

GUJRAT
Roshan, S. (Dr.)

ISLAMABAD
Mirza, Z.B. (Dr.)
Murtaza, T.

JAMSHORO
Abbasi, F.
Afghan, A.A.
Al-Hussain, F.
Ali, M.
Anum, F.
Babar, A.A.

Bai, K.
Bari, A.
Bashir, M.N.
Bhanger, N.
Bhutto, K.A.
Bozdar, M.I.
Bughio, B.A. (Dr.)
Chandio, A.
Das, J.
Dayo, M.S.
Hafeez, R.
Hussain, A.J.
Hussain, M.M.
Jaffery, S.L.
Jakhrani, M.A.
Jakhrani, M.M.
Jamil, M.
Kaka, S.
Khan, N.Z.
Khaskheli, S.
Khattak, N.G.
Khilji, M.J.
Kumari, V.
Luqman, M.
Memon, S.P.
Nawab, M.
Naz, S.
Prince, M.A.
Sajad, W.
Sanam, S.
Sanam, S.
Shah, R.
Shaikh, F. (Dr.)
Sidra-Tul-Muntha
Siyal, S.
Soomro, A.
Soomro, F.D.
Soomro, N.
Soomro, S.
Sultana, R. (Dr.)
Talpur, S.A.
Thebo, A.K.

JHANG
Abbas, S. (Dr.)

KABAL (KPK)
Wahab, A. (Dr.)

KALA SHAH KAKU
Sabir, A.M. (Dr.)

KARACHI
Ahmed, Z. (Dr.)
Ali, S.
Amjad, S. (Dr.)
Ansari, S.
Aslam, S.
Bano, S.
Bibi, S. (Dr.)
Dad, I.
Dilijan, S.
Khan, A. (Dr.)
Khanam, S. (Dr.)
Khawaja, S. (Dr.)
Nasreen, S.
Naz, S.
Rasheed, M. (Dr.)
Riaz, S. (Dr.)
Saher, N. (Dr.)
Sarwar, A.
Savanur, A.
Siddiqui, F.W.

KHAIRPUR (SINDH)
Soomro, F. (Dr.)

KOHAT
Bano, Z.

KOTLI (AJK)
Hussain, A. (Dr.)

LAHORE
Aftab, S.
Ahmad, S. (Dr.)
Ahmad, U.
Ahmed, K.
Aleem, A.
Ali, S.
Ali, Z. (Dr.)
Anjum, F.
Areeba
Arooj, T.
Arshad, A.
Arshad, M.
Ashraf, M.
Aslam, S. (Dr.)
Ayub, M. (Dr.)
Bashir, S. (Dr.)
Bibi, Z.
Dar, N. (Dr.)
Durrani, A.Z. (Dr.)

Faizan, M.
Fatima, N.
Fatima, Q.
Ghazanfar, F.
Haider, A.
Haider, M.A.
Hamid, S.
Hanif, W.
Hassan, H.A. (Dr.)
Hassan, Z.
Hussain, N.
Ijaz, F.
Ijaz, M. (Dr.)
Ijaz, R.
Iqbal, A.
Iqbal, M.P. (Dr.)
Iqbal, Z. (Dr.)
Javaid, F.
Khalid, A.
Khan, A.
Khan, S.Y. (Dr.)
Liaquat, A.
Majeed, A. (Dr.)
Malik, K.
Manzoor, T.
Mobeen, A.
Mubeen, V.
Mushtaq, I.
Nawaz, S.
Naz, S. (Dr.)
Noor ul Ain, H.
Noor-ul-Huda, H.
Qadeer, Q.
Qadir, A. (Dr.)
Qazi, J.I. (Dr.)
Rana, A.N.
Rasheed, S.
Riaz, A.
Rizwana, S.
Sajjad, A.
Saleem, A.
Saleemi, S. (Dr.)
Sarwar, M.Q.
Shah, H.A.
Shaheen, A.
Shahzad, T.
Shakoori, A.R. (Dr.)
Shakoori, F.R. (Dr.)
Sharif, S. (Dr.)
Shaukat, S.
Shokat, S.
Soumble, Z.
Tabassum, I.

Tahir, T.
Tanveer, M.
Tasadduq, R. (Dr.)
Yaqub, A. (Dr.)
Younas, S.
Yousaf, Z.
Zahid, M.T. (Dr.)
Zahra, I.
Zahra, M.
Zaman, A.

MARDAN
Ali, K.
Usman, I.

MIRPUR (AJK)
Abbasi, A.A. (Dr.)
Afzal, N.
Ali, U.
Awais, I.
Farooq, I.
Fatima, A.
Ghani, S.
Iqbal, S.
Jabeen, Z.
Khushal, S.
Kiani, S.
Matloob, A.
Mughal, T.A.
Nawab, S.
Nisar, R.
Razzaq, S.
Shoukat, R.
Waheed, S.
Waqas, Z.
Zafar, H.

MULTAN
Ahmad, S.
Bibi, A. (Dr.)
Hameed, A. (Dr.)
Kausar, N. (Dr.)
Khan, A.

MUZAFFARABAD
Janjua, A.R. (Dr.)
Khalid, M.

OKARA
Chaudhary, F.
Rizwan, M.
Wajid, M. (Dr.)

PATOKI
Abbas, S.
QUETTA
Karim, A. (Dr.)

RAWALAKOT (AJK)
Chishti, M.R.A.
Mahmood, M. (Dr.)
Muhammad, A. (Dr.)
Rahman, Z.
Riaz, S.
Ullah, S.
Yunus, N.

RAWALPINDI
Akber, S.
Ayesha, S.Q.
Baig, R.M.
Bibi, N.
Farid, Z.
Farooq, H.
Gill, S.
Javed, K.
Javed, N.
Kainat, N.
Kashaf, M.
Khan, S.
Mahmood, T. (Dr.)
Naseem, N.
Nawaz, S.
Rais, M. (Dr.)
Sheraz, A.
Waqar, U.
Zaib-un-Nisa
Zamir, K.

SAKRAND (SINDH)
Jalbani, S. (Dr.)
Noor, S. (Dr.)
Odhano, S. (Dr.)

SARGODHA
Ali, A.
Amjad, N.
Ashraf, S. (Dr.)
Hafeez, T.
Khalid, A.
Mehmood, N. (Dr.)
Nauman, M.

SIALKOT
Ehsan, S.

Fargoson, A.
Fatima, T.
Kausar, T.
Rani, M.
Rasheed, F.
Taj, J.

SWABI (KPK)
Ahmad, A.
Ahmad, M.
Ahmad, M.S. (Dr.)
Ahmad, T.
Ahmed, M.
Ahmed, W.
Aliya
Awais, M.
Aziz, F.
Bacha, H.
Hasan, B.
Hassan, S.
Hussain, S.
Khalid
Khan, J.
Khan, M.A.

Maghaz, M.
Mehran, M.
Nawaz, M.
Rahman, A.
Rehman, N.
Riaz, M. (Dr.)
Saira (Dr.)
Sajid, M.
Saleem, D.
Shah, F.A.
Shah, S.
Shahzeb
Tahir, F.
Ullah, A.
Umer, M.

TANDOJAM
Brohi, M.U.
Khatri, I. (Dr.)
Manan, H.
Nahiyoon, S.A. (Dr.)
Rajput, Z.
Solangi, B.K. (Dr.)
Soomro, A.P.

THATTA
Mahar, M.A. (Dr.)

TURBAT (BALOCHISTAN)
Bakhs, B.R.
Kakar, N. (Dr.)
Khan, S.S.
Majeed, Z.
Nabi, G.
Qadir, T.
Waheed, Z.

UTHAL (BALOCHISTAN)
Bano, A. (Dr.)
Habibullah
Rasheed, S. (Dr.)
Siddique, S. (Dr.)

Diversity of Aphids and Assessment of their Potential Natural Enemies in District Sialkot, Punjab, Pakistan

Tasneem Kauser[1], Sajida Mushtaq[1]*, Sadia Maalik[1], Moazama Batool[1], Naheed Bano[2] and Wisha Jaffer[1]

[1]Department of Zoology, Government College Women University, Sialkot
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ABSTRACT

Ecosystem has been found to be an integral part of biodiversity. Predator-prey relationship acts as a driving force in structuring ecosystem whereas, pests are found to be a major reason for developing biotic stress in the agricultural sector. Aphids are found to be one of the serious agricultural pests thus it is necessary to eradicate them to prevent from further damage. The present study was done to determine variation in species of aphids and to determine their selected predators. Sampling was done from the month of February till May, 2021 through crop system of District Sialkot, Punjab, Pakistan. The major crops like wheat, brassica, mustard, rice and sugarcane were selected to sample the aphid species from different available crops. Total 8402 specimens were collected from different crops and were recognized up to species level belonging to the family Aphididae. Total eleven species of aphids were recorded after identificatrion. Shannon wiener diversity indices was applied to determine relative abundance, evenness, species richness and dominance. Tukeys test was performed for further statistical analysis. *Rhopalosiphum maidis* and *Schizaphis graminum* were found to be the most abundant species. Mean predatory efficiency of *Episyrphus balteatus* was maximum when feed on *Schizaphis graminum* as compared to *Ischiodon scutellaris.* On the other hand, predatory efficiency of *Coccinella septempunctata* was maximum when fed on *S. graminum* and minimum when fed on *R. maidis* whereas *Avicularia variegata* mean predatory efficiency was found to be maximum when fed on *R. maidis.* Overall, *C. septumpunctata* has been found to be an important biological control agent for the maintenance and stability of the agro ecosystem.

INTRODUCTION

Biodiversity plays a crucial role to sustain life on this planet by providing fundamental ecosystem services thus they are interdependent on each other. In agro-ecosystems, biodiversity is usually the computation of relative numbers and species of organisms. Insects are the largest and most diverse group of organisms in the world (Ghani and Maalik, 2020). Industrial revolutions have changed the communities and patterns of ecosystem. As a result, many environmental and pests problems have been endangered (Lambers, *et al.*, 2011). The stability of agro

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ecosystem is the result of different interactions among mutually dependent groups of species that coordinates in many ways. The different predators and prey interactions leads to the stability of an ecosystem. In these ecosystems thrashing of a single species strongly affects the entire system and may diminish it (Rana *et al.*, 2019).

Plant insect interactions include extensive variety of relationships like herbivory, insect pollination, and plant provision of food and shelter to insects. The chemistry and structure of plants provides defense mechanisms against insect herbivores, also attracting parasitoids and predators of insect herbivores (Badenes, 2022).

Insects are one of an important creature on earth, they play multifunctional role in the environment by providing different services such as biological indicators, decomposers, pollinators, scavenger, pest, predator in the agroecosystem (Asghar *et al.*, 2022) Aphids belonging to family Aphididae are the major crop pest, included in super family of Aphididae under order Homoptera and placed under the class Insecta of phylum Arthropoda. Aphids are classified under sub order sternorrhyncha and infraorder is aphidomorpha (Dixon and Thieme, 2007). They possessed small size of 0.7 to 7mm in length and known as plant lice

or ant-cows (Ghosh and Singh, 2000). The damage caused by aphids is mostly recorded on cereals crops, shady trees, ornamental fruit, fiber yielding crops and vegetables etc. (Ghosh and Singh, 2000). The colonies of aphids are usually located on the tip of the branches, under the leaves or wherever there is new growth.

Aphids usually acquires on the localized initial infestation but increases rapidly if they are unobstructed. Aphids' transmits various plant viral diseases causing damage to plants by sucking the sap from twigs, leaves, roots or stems. Spotty yellow discolorations appear on the leaves that are attacked by the aphids, usually on the undersides (Singh *et al*., 2015). The leaves may wilt, curl and dry out. Some aphid's species causes swelling or galls of plant tissues plants to form galls in globular form or in spindle shape.

Some species of aphids affect plants by developing galls (swellings of plant tissues which are globular or spindle-formed). The galls usually turn brown and incorporate in any stage of development (Quednau, 2001).

Aphids and their predators are continuously being studied all over the world, in certain regions of Pakistan, but no previous studies were conducted in Sialkot region. Current study demonstrates the diversity of aphids to investigate the feeding potential of selected predators species of syrphids and coccinellids on aphids. Result of present study describe the future implementation of these syrphid or coccinellid species to use as biological control agent in future or in Integrated Pest Management (IPM) in future for sustainable agro ecosystem.

MATERIALS AND METHODS

Study area

Sialkot lies within Punjab Province. It is comprised of four tehsils including Sialkot, Daska, Sambrial and Pasrur (Al-Rashid *et al*., 2021). It has a population of 2.7 million that lives in area of 3016 sq. km (Ghani and Maalik, 2020). The sampling duration was from the month of February 2021 to May 2021. Government College Women University Sialkot, Pakistan was measured as zero point for the selection of sampling sites. Sampling sites were selected randomly and was carried out by setting up an area of 40 km from the zero point. Different locations of District Sialkot were visited to perform sampling such as, Sambriyal, Daska and Pasrur and Sialkot.

Collection of specimens

For collection of specimens two to three fields of each crop were randomly selected. Before starting the sampling, the information was collected about fertilizer application, its type and method of irrigation used in each crop.

The aphids species were sampled by using the Quadrat method from different crops such as corn, sugarcane, wheat, rice and brassica, at different localities of Sialkot, during sampling aphids were inspected on plant materials like as tender leaves, stem, apical portion, flower, fruits twigs, inflorescence, bark and root by using camel hair brush directly from the plants or hand picking. As they are host specific and quadrate of 1 meter square was selected for this collection. Four quadrates, two from center and two from edges. Then, samples were placed in plastic jars and tighten with the rubber bands and in plastic vials of normal width with cotton plug and were brought safely to the laboratory.

Predators of aphids such as Syrphid fly larvae and lady bird beetle's larvae by hand picking method and adults' species were collected by using sweep nets. Insect collecting net made up of common mosquito net cloth, with collecting bag and a wooden handle, used for collecting insects. By sweeping the net on the crop the insect was collected.

Preservation and identification of specimens

Specimen will be preserved in separate glass vials containing preservative solution of alcohol and glycerin in a ratio of 70:30, respectively. Glass/plastic vials will be labelled with date, time, area of sampling and the number of specimens collected (Rana *et al*., 2019). The sticky tag was pasted on each of the collecting vials so that they can be easily identified. The specimens collected were labeled, preserved and further studies were conducted in the laboratory of Zoology Department of GCWU, Sialkot. Collected specimen will be identified up to specie level with the help of the microscope, species were scientifically confirmed by using the taxonomic keys and different online identification keys available on different websites and by applying the available classification-based information in "Introduction to the study of insects" (Kuo *et al*., 2006).

Assessment of predatory potential

Selected predators species were lady bird beetles (*Coccinella septumpunctata* and *Avicularia variegata*) and syrphid fly larvae (*Episyrphus balteatus* and *Ischiodon scutellaris)* were selected based on their high relative abundance in all selected crops. Two species of aphid *Rhopalosiphum maidis* and *Schizaphis graminum* had provided them as a food. These experiments were conducted in the insect cages covered with the mesh net cloth to provide appropriate ventilation and sunlight to make their survival possible. During each experiment 5 to 6 specimens of predators were used. All the predators were provided with enough aphids to satisfy them but not in much excessive amounts. This experiment was carried

out in the Research Laboratory of Zoology department at GCWU, Sialkot and experiment was carried out in month of March.

After 24 h the observations were recorded by analyzing the number of aphids consumed and petri plates kept in insect cages were cleaned again for the next experiment and then counted number of aphids were again offered to predators species. For control group an insect cage was considered as a rearing cage by keeping only aphids in the cage to determine the accurate non predation mortality rate. Aphids provided to predators were always of equal number for making correct comparisons. 20-40 were used for instar larva and their number was increased up to 120-180 by increasing the larval instar. Prey consumption, predator prey interaction and average per day consumption were also recorded on daily basis. The experiment was repeated thrice along with its three replicates.

Statistical analysis

The statistical analysis was performed on the data obtained to determine species richness, diversity and evenness by using diversity indices i.e. The Shannon-Wiener index detected the changings in the abundance of species that are uncommon in a community, and the Simpson index was used to identify the changings that were abundantly distributed among the species of a community (Solow *et al.*, 1993).

Total species number in a habitat were considered as S and No. of individuals of all species were N to determine the species richness. Simpson's index and Shannon diversity index were calculated to support the species richness index.

RESULTS

Total 8402 specimens were collected from different crops and identified up to species level belongs to family Aphididae. Eleven Species were identified in all the areas. *Aphis nerii, Rhopalosiphum maidis, Aphis ochropus, Schizaphis graminum, Aphis spiraecola, Melnaphis sacchari, Melanaphis pyraria, Macrosiphum euphorbiae, Aulacorthum solani, Aphis glycine* and *Aphis fabae.*

Relative abundance

Aphid's fauna was collected from six different crops namely wheat, mustard, corn, brassica, rice and sugarcane. Overall, mustard crop showed maximum relative abundance of aphids whereas with rice crop show minimum diversity and relative abundance was recorded. Brassica crop was rich with respect to aphids species as illustrated in Figure 1C. Maximum relative abundance of 35.53% was recorded in the month of March and minimum abundance of 16.48%

was recorded in May followed by April with 18.69% and February 29.28% (Fig. 1B) Overall, from all selected sites the *R. maidis* appeared with highest relative abundance i.e. 5.30% and *M. sacchari* showed least abundance of 2.77% as indicated in Figure 1A indicated that Sambrial showed high relative abundance of 28.66% and Sialkot showed least relative abundance of 20.95%, followed by Daska 26.11% and Pasrur 24.27%.
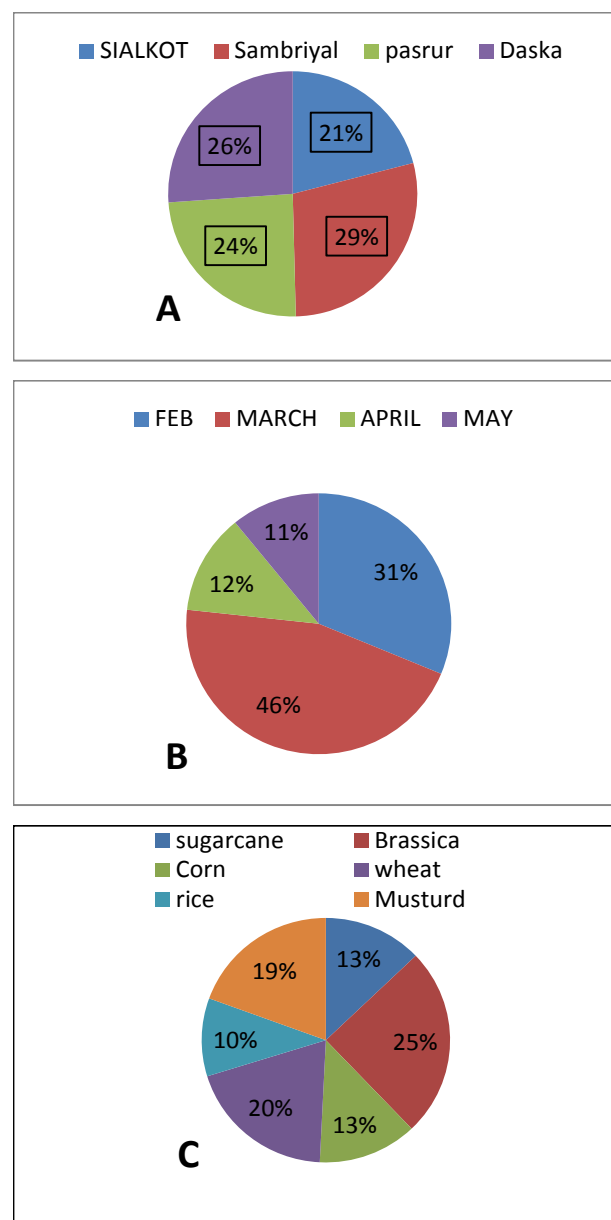
Fig. 1. Percent relative abundance of aphids in different localities (A), during different months (B) in selected crops (C) in Sialkot.
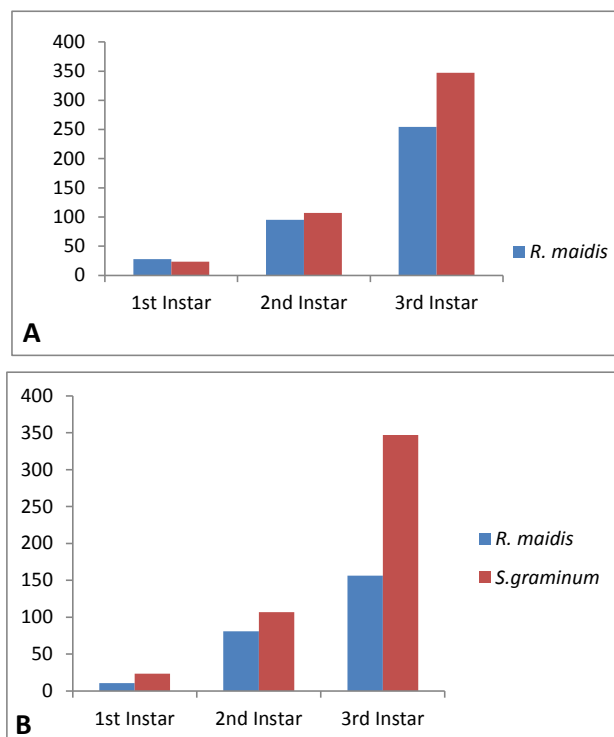
Fig. 2. Predatory potential of *C. septumpunctata* (A) and *A. variegata* (B) on different larval instars.

Feeding potential of predators

Larva of *E. balteatus* consumed the highest mean number of *S. graminum* (215±16.4) followed by that of 204.95±17.04 specimens of *R. maidis* till its pre-pupal stage. Maximum consumption of aphids was recorded by 3rd Instar of larvae when feed on *S. graminum* and *R. maidis* as demonstrated in Figure 2 and Table I.

Maximum number of aphids species *S. gramimun* was consumed by *I. scutellaris* was with consumption of 185.05+17.58 specimens and low number of *R. maidis* with consumption of 174.23+17.34 specimens.

C. septempunctata consumed high number of *S. graminum* by feeding on 495.3+48.6 specimens and low number of *R. maidis* by feeding 361.3+20 specimens. *A. variegata* consumed highest number 324+8.6 species of *R. maidis* and lesser number 322.3+27.35 specimens of *S. graminum* (Table I).

Shannon- wiener diversity indices was performed on the data obtained from selected crops. This diversity index determined the richness and evenness of individual aphid species in selected agro ecosystem of Sialkot. Richness (H`) was highest in corn (2.27) and sugarcane (2.17) followed by corn (2.28), Brassica (2.17), rice (2.043). All crops showed significant difference in results having p value <0.05 except sugarcane vs wheat showed insignificant results having p values > 0.05 i.e. 0.863 as shown in the Table II.

Table I. Predatory efficiency of *E. balteatus* and *Ischiodon scutellaris* in different larval instars on two different species of aphids.

Larval instars	*E. balteatus*		*I. scutellaris*		*C. septumpunctata*		*A. variegata*	
	S. graminum	*R. maidis*	*R. maidis*	*S. graminum*	*R. maidis*	*S. graminum*	*S. graminum*	*R. maidis*
1st Instar	30±2.88	28.33±3.03	19.86±3.70	21.9±3.98	25.7±1.69	25±2.94	12±2.83	15.3±1.7
2nd Instar	65±6.45	62.02±6.97	51.15±5.85	55.87±6.15	114±10.2	88±9.62	101.7±8.1	159.3±12.4
3rd Instar	120±7.07	114.6±7.04	103.22±7.79	107.28±7.45	221.7±10.5	180.7±9.4	282.3±8.34	324±8.6
Grand total	215±16.4	204.95±17.04	174.23±17.34	185.05±17.58	361.3±20	495±48.6	322.3±27.35	324±8.6

Table II. Shannon diversity index of aphid distribution in selected crops of district Sialkot.

Crops	N'1	H1	E1	N'2	H2	E2	Df	t test	p value
Sugarcane vs rice	1389	2.17	0.79	1247	2.043	0.701	2444	4.88	0.5
Sugarcane vs corn	1389	2.17	0.79	1416	2.27	0.88	2529	5.53	0
Sugarcane vs Brassica	1389	2.17	0.79	2252	2.169	0.842	2119.2	2.57	0.024
Sugarcane vs wheat	1389	2.17	0.79	2089	2.173	0.789	2964	0.171	0.863
Rice vs corn	1247	2.043	0.701	1416	2.27	0.88	2003	10.11	0
Rice vs Brassica	1247	2.043	0.701	2252	2.169	0.842	1666	3.92	0
Rice vs wheat	1247	2.043	0.701	2089	2.173	0.789	2262	5.39	0
Corn vs Brassica	1416	2.27	0.88	2252	2.169	0.842	2769	10.65	0
Corn vs wheat	1416	2.27	0.88	2089	2.173	0.789	3494	6.08	0
Brassica vs wheat	2252	2.169	0.842	2089	2.173	0.789	3572	2.87	0.004

Table III. Shannon diversity index of aphid distribution during selected months.

District	N'1	H1	E1	N2	H2	E2	Df	T test	P value
Feb vs. March	1693	2.285	0.893	2986	2.338	0.942	2672	4.168	3.158
Feb vs. April	1693	2.285	0.893	2460	2.328	0.932	3062	3.158	0.001
Feb vs. May	1693	2.285	0.893	1385	2.324	0.929	3077	2.592	0.009
March vs.April	2986	2.338	0.942	2460	2.327	0.932	5004	1.129	0.258
March vs. May	2986	2.338	0.942	1385	2.324	0.929	2418	1.211	0.2257
April vs. may	2460	2.327	0.932	1385	2.324	0.929	2821	0.275	0.783

Table IV. Shannon Diversity index applied on aphid diversity in different tehsils of District Sialkot.

District	N1	H'1	E	N2	H'2	E2	Df	t test	p value
Sialkot vs Sambrial	1760	2.274	0.883	2408	2.299	0.906	3462	1.723	0.084
Sialkot vs Pasroor	1760	2.274	0.883	2040	2.334	0.103	3170	4.213	2.583
sialkot vs Daska	1760	2.274	0.883	2194	2.339	0.942	2943	4.681	2.98
sambrial vs Pasroor	2408	2.299	0.906	2040	2.334	0.938	4447	2.93	0.003
sambrial vs Daska	2408	2.299	0.906	2194	2.334	0.942	4480	3.473	0
Pasroor vs Daska	2040	2.334	0.938	2194	2.339	0.942	4128	0.404	0.685

The highest T- value found between Feb and March (4.168) and lowest value (0.275) between April and May. Feb vs. April and Feb vs. May have p value <0.05 showing significant results while all other months show insignificant results having p values >0.05 as demonstrated in the Table III.

Shannon Weiner showed that Daska possess highest H` value i.e. 2.339 while, Sialkot indicated least value of H` (2.29). Highest Evenness found from Daska 0.942 and lowest value from Pasrur 0.103. The T value was highest for Sialkot vs. Daska 4.681. Sammbriyal vs. Daska and Sammbriyal vs. Pasrur have significant results showing that both groups are more similar as p value is less than 0.05 while all other groups showed insignificant results indicating that the groups are different and highest p values were found between Sialkot vs. Daska (2.98). This is indicated in Table IV.

DISCUSSION

Aphids are common pest known as plant lice with a wide range of the hosts. Total number of aphid's specimens in whole sampling period was found to be 8402. Eleven different types of aphids were collected during whole sampling period that belongs to different genera named as *Aulacorthum solani, S. graminum, R. maidis, Macrosiphum euphorbiae, Aphis glycines, Melanaphis pyraria, Melnaphis sacchari, Aphis spiraecola, Aphis ochropus, Aphis nerii* and *Aphis fabae*.

In the present study most of the species evaluated belonged to genus *Aphis.* Species of genus aphis are abundant species of corn and beans as stated by Mcdonald *et al.* (2003). *S. graminum* was second most abundant species found in present study. It causes serious damage to wheat crops and more preferably found in wheat according to Khan *et al.* (2012) but these results are not according to the prior studies performed on this particular species as in earlier studies their abundance were indicated in the crop fields of sugarcane and wheat relatively less in number in case of wheat crop. Least diversity of *Aphis nerii* was indicated in the whole sampling period from all selected crops but considered to be as a significant pest according to Agrawal and Singh (2005).

Alternation in the climatic conditions, rise in temperature, environmental fluctuations, and atmospheric changings cause serious effect on the invasive pests and aphids are greatly affected by these changings and most abundant fauna was recorded in the season of winter particularly in the months of February, March followed by April and May. As, the temperature starts to increase the aphids population starts to decrease i.e. least fauna was present in fall and summer. Similar results were shown by Sepulveda *et al.* (2021). Hulle *et al.* (2010) also described that the change of temperature resulted in decline of aphid population which is in accordance with the current results. No significant difference was found in aphids fauna of between Feb and March, but significant difference was found in April and May.

Most of the aphids species were also found in *Brassica* crops belonging to genus *Rhopalosiphum* as *R. maidis* was present in abundance in all crops as shown by their maximum number in the field and least number of aphid species was present in rice crops and maximum number was shown by *Brassica*, wheat and sugarcane respectively. Severe infestation in of aphids the crops greatly affected the growth of the plants resulting in the serious loss. The studies are in accordance with the Chaudhary *et al.* (2009).

District Sialkot showed great abundance of Aphids species and mostly aphid fauna was found in Brassica and wheat crops these results are in accordance with Ghani and Maalik (2020) which demonstrated that 37 % of Aphids species among all other insects are found in District Sialkot and in wheat crop most abundant species were *S. graminum* and *R. maidis*. *Melanaphis sacchari* and *Melanaphis* pyraria's maximum number reported in sugarcane that showed their affinity towards sorghum. Bowling *et al.* (2016) confirmed that *melanaphis sacchari* is an invasive pest of sugarcane species and causes 20 to 50 % of yield losses. *Macrosiphum euphorbiae* was most abundant in corn and secondly abundant species were *S. graminum* in corn. *Aphis ochropus, R. maidis and M. euphorbiae* were relatively more in rice field. *Aphis fabe* presence was mostly indicated in corn field.

Predators feeding potential on Aphids

Syrphid larvae and leady bird beetles have potential to suppress the aphids population as aphids density started to decrease in the presence of these predators so they act as one of the most important biological control agents. Larvae of two species of syrphid named as *E. balteatus* and *I. scutellaris* and two species of lady bird beetles i.e. *Adigona variegata* and *C. septumpunctata* were selected to check their predatory potential against two aphid species i.e. *S. graminum* and *R. maidis*. According to Faheem *et al.* (2019) *E. balteatus* and *I. scutellaris* was directly related with decline of aphid population.

Syrphid larvae of *E. balteatus* and *I. scutellaris* was abundant in the month of march. As, spring season is a flower blossoming period in Pakistan as Lambers *et al.* (2011) revealed that hoverflies are flies of spring season. Present study revealed that aphid consumption increased as instars larvae of syrphid increases, and these results are according to the findings of Nelson *et al.* (2012) who showed that early instar larvae can consume aphids that greatly increases the consumption rate with the later stages of larval instars.

Survival of *E. balteatus* larvae was recorded maximum when fed on *R. maidis* and minimum when fed on *S.graminum*. Maximum survival of *Ischidon* larvae was observed when they fed on *R. maidis* and minimum found

on when fed on *S. gramimun*. Mean predation of *Ischidon* larvae is slightly more than *E. balteatus*. Same results were indicated by Lambers *et al.* (2011) that showed predation rates differ according to the different aphid species.

More survival rate of larval instars was recorded when *C. septempunctata* fed on *S. graminum* species and when the larvae feed on *R. maidis* the survival percentage was less. These findings are according to the previous findings of Johnthan and Lundgren (2005). Percentage of survival was comparatively low in *A. variegate* than *C. septempunctata*, 79% when recorded in *S. graminum* and 78% was observed in *R. maidis*. The same results were reported by Maharjan *et al.* (2018) who compared the feeding efficacy of two species of beetles *C. septempunctata* and *A. variegata*. *C. septempunctata* consumed more number of aphids than *A. variegate* because of its larger size and more feeding requirements. The 4th instar larvae of lady bird beetles are voracious feeders of aphid. Larvae of lady bird beetles managed aphids in better way and are voracious feeders (Lucas, 2005).

The present results concluded that lady bird beetles proved to be more efficient predators followed by syrphid flies.

From all above discussion, it is stated that all aphid species belong to different croplands and ecological niches according to their preferences for feed. Brassica crop was found to be rich in aphid fauna during the months of February and March. The suitable season is spring for aphid's fauna. Lady bird beetles especially *C. septumpunctata* was proved to be more efficient predators as compared with other species of beetles and also showed more predation rate as compared with syrphid larvae. Ladybird beetles thus, can be used as efficient bio control agent against aphids.

Developmental stages of prey

Mean developmental period of *C. septumpunctata* lady bird beetles is 31.56±2.21 day when fed on *S. graminum* and *R. maidis*. Mean developmental period of *A. variegata* lady bird beetle is 28.57±2.55. Mean developing time of *I. scutellaris* 26.46±2.3 days. *E. balteatus* development in total period is 31.56±2.21. Debraj and Singh reported the correlated results and Muzammil *et al.* (2008) reported similar findings, stating that pupal stage depending on food supply and temperature, can last anywhere from 3 to 12 days.

CONCLUSION

Eleven different species of aphids were found from different selected sites of Sialkot. Higher abundance of aphids was found in Brassica and sugarcane and, lowest

population was recorded from rice crops. Maximum fauna of aphids was recorded in the month of March followed by February, April, and May respectively. *R. maidis* was most abundantly present species followed by *Schizaphis graminum.* Among syrphid predators *Episyrphus balteatus* was more efficient predator than *Ischidon scutellaris* while*, C. septumpunctata* was more efficient as compared to *A. variegata*. *S. graminum* was most preferred species of *E. balteatus*, whereas *I. scutellaris* preferably consumed *R. maidis*. Same predatory preference was recorded in *C. septumpunctata* and *A. variegata* regarding aphid species*.*

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Toxic Effects of Antihypertensive Drug Propranolol Hydrochloride on Hormonal Indices of *Oryctolagus cuniculus*

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ABSTRACT

Propranolol hydrochloride is a synthetic, non-selective beta-adrenergic receptor blocking agent, appears to be an effective treatment for hypertension and myocardial infraction. At dosages greater than required for beta blockade, propranolol also affects the cardiac output and hormonal parameters. Twenty healthy mature male New Zealand rabbits (*Oryctolagus cuniculus*) were orally intoxicated with propranolol through 1cc syringe. Group A was considered as the control group and served with food and water only while group B was the experimental group intoxicated with propranolol at the dose of 05 mg/kg body weight regularly for 30 days. TOSOH AIA system Hormonal analyzer (Made in Japan) was used for analysis of the hormonal indices. We found that propranolol caused adverse effects like weakness, restlessness and diarrhea in the treated rabbits. Also, an increase of 55.85%, 4.45671% and 48.6239% in the level of TSH, estrogen and progesterone, respectively was found. While a decrease of -28.8557% in the level of T3, -5.87276% in T4 and -22.3762% in testosterone level. To conclude the study shows that propranolol has adverse effects on the thyroid as well as on sex hormones of treated rabbits.

INTRODUCTION

Propranolol is an anti-hypertensive drug, belongs to beta blockers and is found as a white, stable and crystalline solid which is readily soluble in water and ethanol. Its molecular weight is 295.80. It specifically competes with beta-adrenergic receptor agonist agents for available receptor sites. When access to beta-receptor sites is blocked by propranolol, the chronotropic, inotropic, and vasodilator responses to beta-adrenergic stimulation are decreased proportionately. At dosages greater than required for beta blockade, propranolol also exerts a quinidine-like or anesthetic-like membrane action, which affects the cardiac action potential. Right from reduction in postmyocardial mortality to control of anxiety in performers, propranolol plays an important role in a plethora of medical conditions.

Moreover, propranolol treatment has been found to be cost-effective when compared to other corresponding treatment options for individual indications (Rehsia and Dhalla, 2013). Other applications of propranolol in several non-cardiovascular conditions including migraine, essential tremors, anxiety, portal hypertension, hyperthyroidism, and pheochromocytoma have been recognized. Propranolol has also been shown to be effective in pediatric migraine and coronary heart problems. In angina pectoris, propranolol generally reduces the oxygen requirement of the heart at any given level of effort by blocking the catecholamine-induced increases in the heart rate, systolic blood pressure, and the velocity and extent of myocardial contraction. Propranolol may increase oxygen requirements by increasing left ventricular fiber length, end diastolic pressure, and systolic ejection period. Just like other medications, predominantly beta-blockers are metabolized in the liver (Shin and Johnson, 2007). A quarter of the ingested drug reaches to the systemic circulation due to the first-pass metabolism in the hepatic circulation. As the propranolol is metabolized by hepatic enzymes and excreted through the renal system, so the prescribers should proceed with cautions when prescribing it to the patients with known hepatic and renal problems. Furthermore, dosages may need to be adjusted to avoid toxicity resulting from the inability to metabolize or clear

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the medication from the body properly.

As still no adverse effects of propranolol on endocrinology has been reported, the present study was aimed to determine its toxic effects on thyroid and sex hormones.

MATERIALS AND METHODS

Experimental plan

Male New Zealand rabbits (*Oryctolagus cuniculus*) were used as an experimental animal and as a human model. Twenty healthy mature rabbits were procured form the local market and grouped in to two groups A and B having ten rabbits in each group. Group A was served as the control group while group B was the experimental group. Rabbits were kept in the Zoology Lab, Women University Swabi at normal of temperature 25°C with relative humidity of 65±5% and with proper housing conditions. The rabbits were housed in clean and well-ventilated animal stainless steel cage having many compartments. Food such as fresh vegetables and water was provided at *ad libitum*. The drug propranolol was orally given to the rabbits through 1cc syringe. Group A was considered as the control group and fed with fresh food and water only while group B was the experimental group intoxicated with propranolol at the dose of 05 mg/kg body weight. Rabbits received the dose regularly for 30 consecutive days.

At the end of experimental period the blood samples were collected in the gel tubes from the ear marginal vein by making a cut. After blood collection, the serum was separated from the blood by centrifugation at 3000 rpm and stored in specialized viols in the refrigerator at 4°C for hormonal assays to be performed. TOSOH-AIA system analyzer (Made in Japan) was used for the analysis of hormonal indices.

Statistical analysis

The collected data was analyzed via one- way analysis of variance (ANOVA) and the mean and standard error of mean values were compared by using the SPSS version (26).

RESULTS

A dose of 5 mg/kg body weight of antihypertensive drug was administered orally to male New Zealand rabbits (*Oryctolagus cuniculus*) for thirty consecutive days. After the stipulated time period, changes in the sex hormones as well as in the thyroid hormones were recorded.

Table I shows that the Mean±SEM of thyroid stimulating hormone (TSH). In the control group the Mean±SEM was 3.86±0.97 mU/L while in the propranolol

intoxicated group it was found to be 6.016±2.12 mU/L such that an increase was found in the TSH level. An increase in the estrogen level was also found. In the control group the Mean±SEM value for the estrogen was 47.12±2.99 while in the propranolol intoxicated group it was 49.22±2.31. Progesterone level was also increased in the treated group. In the control group the progesterone level was 2.18±1.45 while in the treated group the Mean±SEM value was 3.240±1.11. The level of T3, T4 and testosterone was decreased in comparison with the intoxicated group. T3 in the control group was 4.02±1.265 while in the treated group it was 2.86±0.11. T4 was having the value of 12.26±0.22 in the control group while in the test group the Mean±SEM value was 11.54±0.27. The value of testosterone in the control group was 2.02±0.13, while in the propranolol intoxicated group it was 1.568±0.01.

The other adverse effects like weakness, restlessness and diarrhoea have also been reported in the rabbits intoxicated with propranolol.

Table I. Blood hormonal indices after thirty days oral intoxication of antihypertensive drug propranolol with a dose of 5 mg/kg to *Oryctolagous cuniculus*.

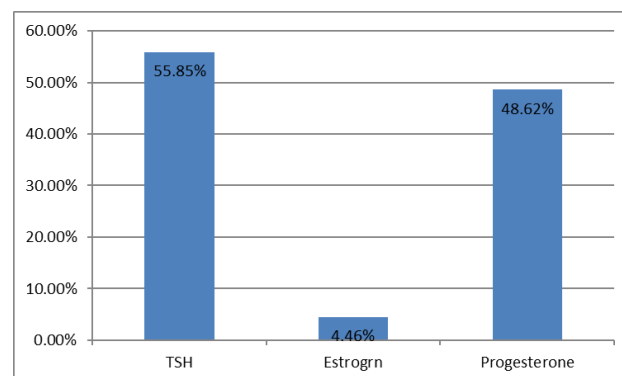| S. No	Parameters	Control (n = 3)	Propranolol (n = 3)	Percentage
1	TSH	3.86±0.97	6.016±2.12	55.85% ↑
2	Estrogen	47.12±2.99	49.22±2.31	4.45% ↑
3	Progesterone	2.18±1.45	3.24±1.11	48.62% ↑
4	T3	4.02±1.265	2.86±0.11	-28.85% ↓
5	T4	12.26±0.22	11.54±0.27	-5.87% ↓
6	Testosterone	2.02±0.13	1.57±0.01	-22.37% ↓

Fig. 1. Percentage increase of TSH, estrogen and progesterone.

Figure 1 shows that thyroid stimulation hormone (TSH) in comparison with control group increased up to

55.85% in experimental group. Estrogen increased up to 4.45% in propranolol intoxicated group while progesterone increased up to 48.62% in the treated group.
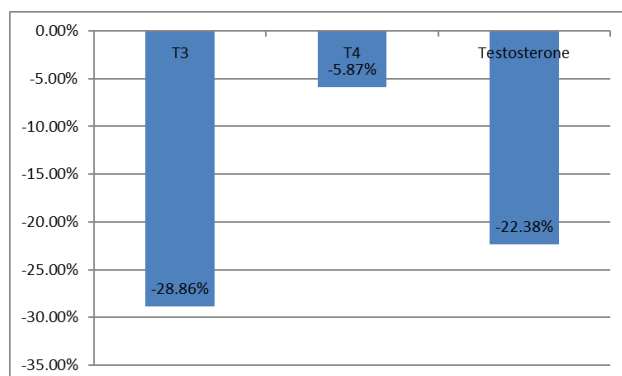

Fig. 2. Effect of propranol hydrochloride on percentage decrease of T3, T4 and testosterone.

Figure 2 shows that T3, T4 and Testosterone level was decreased in the propranolol intoxicated group i.e. a decrease of -28.85%, -5.87% and -22.37% was found respectively. The negative signs indicate decrease in these parameters.

DISCUSSION

In the current study the rabbits were analysed for the adverse effects of propranolol on hormonal indices. It was found that Thyroid stimulation hormone (TSH) in comparison with control group increased up to 55.85% in experimental group. The results of the current study herein showed the TSH-lifting by β-adrenergic receptor blockers and hence proving the association between β-blockers and thyroid functions. This might indicate an increase in TSH secretion. Second, the changes in the thyroxine (T4) and T3 values could influence serum TSH levels in negative feedback.

Ten patients with euthyroid multinodular goitre received propranolol (Inderal Retard 160 mg daily) for 4 weeks in order to investigate the effect on TRH, TSH and serum T3 levels. It was that, Serum T3 decreased significantly after 2 and 4 weeks of propranolol intoxication while T4 increased significantly. The TSH response to TRH increased significantly during the administration of propranolol. Mean delta TSH basally was 1.0, range 0-3.9 mU/l, and mean delta TSH after 4 weeks of propranolol was 2.4, range 0-9.3 mU/l. A strong correlation was found between the increase in delta TSH and the decrease in serum T3 after 4 weeks of propranolol administration. The data suggested that T3 is an important factor in the

impairment of the TSH response to TRH. These findings support the concept of subclinical hyperthyroidism in 'euthyroid' multinodular goitre with autonomous function (Elte *et al*., 1982). The effect of propranolol on thyroid status was investigated by administering the drug in 2 therapeutic doses (80 mg blood weight and 120 mg blood weight) to 8 healthy volunteers and serially measuring total and free thyroid hormones and their major binding protein. Mean free T3 fell by 1.2 pmol/l (P< 0.05) whilst mean free T4 and mean rT3 rose by 3.3pmol/I (P< 0.001). Despite the changes in the free hormone levels there was no significant change in TSH (Wilkins *et al*., 1985).

In the current study estrogen increased up to 4.46% in propranolol administered group while Progesterone increased up to 48.62% in the treated group, showing that Propranolol effected the adrenal cortex as well as the testis of the rabbits due to which both the hormones level was increased. High level of progesterone in males influences spermiogenesis, sperm capacitation/ acrosome reaction and testosterone biosynthesis in the Leydig cells. High progesterone levels are associated with family history of premature coronary artery disease in young healthy adult men (Osadnik *et al*., 2019). Similar study reported by Re *et al*. (1993), in which the effects of a 3-day intramuscular administration of clenbuterol (25 µg/Kg), propranolol (12 mg/kg), clenbuterol (25 µg/kg) plus propranolol (12 mg/Kg) and estradiol (0.5 µg) upon the female reproductive system were investigated in immature sprague-dawley rats. Clenbuterol and estradiol treatments induced a significant increase in uterus weight and in relative uterus weight, whereas in the groups treated with propranolol and clenbuterol plus propranolol no differences were detected. The uterine estrogen receptor levels were significantly increased by clenbuterol administration. In the rats dosed with propranolol and clenbuterol plus propranolol, no modifications occurred in estrogen receptor concentrations. Uterine progesterone receptors were not significantly affected by any of the considered treatments. Data obtained indicate that clenbuterol treatment induces an increase in uterus weight and in estrogen receptor levels and these effects are regulated by acute beta-adrenergic stimulation.

In another study, female sprague-dawley rats were treated intramuscular with 0, 2.5, 25, and 50 micrograms of clenbuterol HCl/kg of body weight/d for 21 days. In all treated rats, significant increase in body weight gain (P < 0.05) and improvement in feed conversion ratio (P < 0.05) were recorded. Hydrometra was observed in the uterus of treated rats, and histologically, it was possible to see dilatation of luminal glands and ovarian alterations. Clenbuterol treatment induced significant (P<0.05) increase in uterine estrogen receptor concentration of rats treated with the 2 higher doses. Treatment apparently

failed to enhance the rate of oxidative and conjugative biotransformation, except for glucuronidation of p-nitrophenol (P < 0.05). Data showing that high doses of clenbuterol affect the female reproductive system of rats, almost in part, estrogen-like modifications, but probably by a different mechanism of action correlated to intense adrenergic stimulation (Re *et al.*, 1993).

The effects of jugular infusions of adrenaline and the β-adrenergic receptor antagonist propranolol on plasma concentrations of progesterone and oxytocin were examined at two different stages of the caprine estrous cycle in the goat. Adrenaline (25 µg.kg−1h−1) significantly (P < 0.05) increased oxytocin secretion on day 3 and day 10 of the cycle (estrus= day 0); progesterone concentrations were significantly (P < 0.05) elevated on day 10 alone. Propranolol had no effect on progesterone secretion yet significantly (P < 0.05) reduced oxytocin concentrations on day 3. The results suggested that there may be neuroendocrine involvement in the regulation of luteal oxytocin secretion.

In the current study, T3 level was decreased to -28.8557% while T4 was decreased to -5.87276% in the Propranolol intoxicated group in comparison with the control group showing that membrane-stabilizing activity of propranolol alters the thyroid physiology and hence the level of TSH was decreased. The use of propranolol may be associated with hypothyroxinemia. The statement is supported by the current study. The propranolol is a non-selective beta blocker, also utilized in states of hyperthyroidism and thyroid storm preferentially due to its effect of blocking the peripheral conversion of inactive T4 to active form T3. Or it might be possible that propranolol blocks the activity of monodeiodinase type I, which decreases peripheral conversion of T4 to T3, i.e., propranolol's effect on the reduction of T4's metabolism through the inhibition of monodeiodinase type I. On the other hand, in a study, the thyroid hormone levels were analysed during, and for 6 days after, treatment with propranolol (160 mg/day, 4-8 weeks) in five hyperthyroid patients and six normal volunteers. In hyperthyroid patients' triiodothyronine (T3) and free triiodothyronine index (FT3I) rose to peak levels 60 h after propranolol withdrawal without significant change in thyroxine (T4) or thyopac-3 test. In normal subjects no significant change in thyroid hormone levels was seen during the treatment of propranolol. The rise in T3 and FT3I coincides with, and may contribute to, the rebound increase in adrenergic activity after propranolol withdrawal in hyperthyroidism while the decrease might be due to decrease in the adrenergic activity (Ross *et al.*, 1980). Similar study was also conducted by Jones *et al.* (1981), in which the serum thyroid hormone concentrations were measured before and during 6 months treatment with propranolol (160 mg/day) in eight patients with T3 (triiodothyronine) toxicosis. Serum total T3 concentrations showed a significant (p less than 0.01) and sustained decrease to approximately 80% of pre-treatment values. Six patients however, remained clinically and biochemically hyperthyroid.

In another study propranolol alone was given to sixteen hyperthyroid patients, and concomitantly with thyroxine therapy to ten hypothyroid patients. Following treatment of the hyperthyroid group for 1-2 weeks there was a significant decrease in serum triiodothyronine (T3) which correlated with the plasma propranolol steady state concentration. The serum reverse T3 (rT3) rose significantly. One patient with T3 toxicosis went into remission. The reduction in serum T3 was maintained in six patients receiving propranolol for more than 1 month. In the hypothyroid group the mean serum T3 level achieved with 0.15 mg thyroxine per day was significantly lower than in a control group who did not receive propranolol. In five patients following propranolol withdrawal there was a significant increase in T3 level, a fall in rT3 and TSH level, and weight loss. Propranolol may therefore have a clinically significant and direct action on the peripheral conversion of thyroxine to T3 and rT3 (Feely *et al.*, 1979). In a study, concentrations of total and free thyroid hormones and thyroid hormone binding proteins were measured in thyrotoxic and euthyroid subjects treated with propranolol. In the thyrotoxic group, total triiodothyronine (T3) concentration fell after propranolol therapy, suggesting an effect of the drug on the peripheral conversion of thyroxine (T4) to T3. In euthyroid subjects, a rise in circulating concentrations of free T4 and reverse T3 (rT3) was observed, while only a small decrease in free T3 was evident. Thyroxine binding globulin (TBG) concentration fell during propranolol treatment while thyroxine binding prealbumin (TBPA) concentration rose. The changes observed in the euthyroid state are consistent with inhibition of peripheral deiodination of T4 and rT3; an additional effect of propranolol on binding protein metabolism was evident (Franklyn *et al.*, 1985).

Another study was conducted on propranolol in which propranolol decreases plasma T3 and increases plasma rT3 in a dose-dependent manner due to a decreased production rate of T3 and a decreased metabolic clearance rate of rT3, respectively, caused by inhibition of the conversion of T4 into T3 and of rT3 into 3,3'-T2. This inhibition of 5'-deiodination is not secondary to inhibition of thyroid hormone transport across the plasma membrane. Propranolol and its major metabolite, 4-hydroxypropranolol, are not directly responsible for these effects, but an unidentified metabolite of propranolol might be involved. beta-blockers ameliorate clinical

symptoms and signs of thyrotoxicosis independent of the decrease of plasma that is confined to beta-blockers with membrane-stabilizing activity, such as propranolol and alprenolol. The decrease of plasma T3, however, appears responsible for some of the metabolic responses to beta-blockers (Wiersinga,1991). Propranolol blocks the activity of monodeiodinase type I, which decreases peripheral conversion of T4 to T3. Propranolol increases the serum reverse T3 concentration with lesser changes in other serum thyroid hormone levels compared to metoprolol (Murchison et al., 1979).

In the current study, the testosterone level was decreased to -22.3762% in the propranolol administered group. It means that there are possible relationships between the Propranolol and the hormones due to which the Propranolol induced changes in anterior pituitary and related hormones. Low level of testosterone that was observed in the current study may be due to the depressant effect of beta-adrenergic blocker on Leydig cell testosterone production the result was also supported by Rosen et al. (1988). Similar study was conducted to investigate beta-blocker effects on sexual function by means of physiological (NPT), subjective, and hormonal measures. Four beta-blockers with different ancillary properties (atenolol, metoprolol, pindolol, propranolol) were evaluated in a placebo-controlled, double-blind, Latin-square design. Thirty healthy male volunteers received, in counter balanced order, each of the four drugs and 1 week of placebo testing. Significant drug effects on both total and free testosterone were found during treatment with all four beta-blockers, although it appeared that the non-selective drugs (pindolol, propranolol) were associated with the greatest reduction in testosterone. Inspection of individual records, however, suggested that some subjects may be especially vulnerable to sexual dysfunction in association with propranolol Rosen et al. (1988).

Treatment of eight healthy males with propranolol (80 mg twice daily) for 6 weeks resulted in a significant reduction in overnight plasma levels of prolactin and LH. Plasma testosterone levels were found elevated whilst GH and cortisol were unchanged by such treatment. GH, cortisol, prolactin, and testosterone plasma levels all showed time dependent changes: propranolol treatment significantly altered the time course of cortisol but not of the other hormones. In addition, the lowered plasma prolactin levels may directly contribute to the hypotensive action of propranolol (Dart, 1981). Same study was conducted by (Lewis et al., 1981) in which the effect of single doses of two beta-adrenoceptor blocking agents such that propranolol (given 80 mg orally) and acebutolol (given 200 mg orally) were analysed. Plasma levels,

GH, prolactin, follicle stimulating hormone, Luteinising hormone, Cortisol and testosterone have been studied in the seven healthy male volunteers. Results showed that acebutolol has significantly reduced the prolactin and follicle stimulating hormone level, but did not alter the levels of the other hormones. Propranolol has significantly reduced follicle stimulating hormone and testosterone level, while significantly increased the level of cortisol, but caused no change in the other hormones. Prolactin, luteinising hormone, testosterone and cortisol showed a significant variation with time indicating the existence of a diurnal rhythm in the pattern of their secretion. There was a significant inter-subject variability in all the hormones.

Propranolol, a non-specific β-adrenergic receptor blocker has been proposed as an effective agent in the management of cardiac disease more specifically in males. Similar study was conducted aiming to observe the effects of propranolol on serum level of testosterone. Fifty-six adult male rats were divided into four groups control and treated were used in the study. 0.5ml injection of distilled water to control and 0.5 ml injection of distilled water containing propranolol 1mg, 2mg and 4mg kg-1 body weight daily intraperitoneally for sixty days was given to treated rats. Serum testosterone and LH hormones were assayed after treatment and recovery period each of sixty days. The change in body weight was similar in control, treated and recovered rats. A significantly lower serum testosterone level ($p < 0.05$) was found in 4mg treated rats when compared with controls. Serum luteinizing hormone also showed significantly lower level ($p < 0.05$). Serum level of both hormones was reversible after a recovery period of sixty days (Naveed et al., 2007). Suzuki et al. (1988), also found that patients taking 50–100mg atenolol experienced sexual dysfunction and reduction of serum level of testosterone hormone. The adrenergic agonistic drugs stimulate the testicular testosterone release from the testis of immature golden hamster via interaction with adrenergic receptors. Significant low serum level of testosterone that was recorded in 4mg propranolol treated adult rat in this study may possibly be due to the direct beta adrenergic antagonistic effects of propranolol on testosterone production through the disruption of adrenergic-stimulated cAMP transduction path way. The low blood testosterone level observed in rat treated with 4mg propranolol in present study was in agreement with the result of El-Syed et al. (1998), in which there was significant decreased blood level of testosterone when propranolol was given at a dose of 15 mg/ kg body weight orally for sixty days. Moreover low level of testosterone observed in the present study is not consistent with the result of Biswas et al. (2001). It was reported that atenolol inhibited the action of noradrenaline on testicular Leydig

cell hyperactivity in cadmium treated rat.

CONCLUSION

Propranolol caused toxicity in different organs and has adversely affected the sex as well as the hormonal Parameters through β-adrenergic blockade. Weakness, restlessness and diarrhoea have also been reported in the rabbits intoxicated with propranolol. Although propranolol is preferably used in hypertensive patients but caution should be exercised prior to use.

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IRB approval
The research proposal was reviewed and approved by the internally Research Project Committee of Women University Swabi.

Ethical statement
All the ethical issues about the animal experimental used was addressed according to the international standards. Every possible care was taken about the animal handling.

Statement of conflict of interest
The authors have declared no conflict of interest.

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Skin Diseases in Different Fish Species of Indus River at Taunsa Barrage, Pakistan

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ABSTRACT

This study was conducted to investigate the skin infections in fishes of Indus River at Taunsa Barrage Southern Punjab, Pakistan for the period of one year (September 2013 to August 2014). A total of 2249 specimens of 70 different species were sampled out of which, 1595 (71%) specimens belonging to 35 species were found to be affected by various types of skin infections. Both commercially important and non-commercial fish species (44% and 27%) were found to be suffering from various skin infections. Among commercially important species; *Systomus sarana* (41%), *Mastacembelus armatus* (37.5%) and *Wallago attu* (33%) were highly affected while *Oreochromis niloticus* (2.9%) were the least affected species. Similarly, *Colisa lalia* (25%), *Xenetodon cacila* (20%) and *Puntius chola* (10.5%) were more affected while *Gadusia chapra* (1.5%) were least effected among the non-commercial fishes. Less skin infection in exotic species *O. niloticus* is another potential danger for local fish fauna of the River. The prevalence of skin infections was at its peak in November (49%) followed by October (27.9%) and February (21.3%) in commercial fishes, while non-commercial fishes were highly affected in March (30%), January (20%), and December (19.3%). Multiple infections, like damaged eyes, ulcer disease, fin rot and tail rot were found in affected specimens. These infections may be due to aquatic pollution, predation, parasitic and microbial attacks on fishes. The highest rate of skin infection in winter months may be attributed to large population density in less water current of the River. The alarming situation of fish health status in the study area, points to the urgent attention of the authorities to protect the diversified fish fauna from any future disaster.

INTRODUCTION

The occurrence of various type of diseases in fish, is one of the most common problem of the aquaculture industry. Many factors promote diseases in fish, but one factor is more important and it is the interaction between pathogens and environment. Stressful environmental conditions affect fish's well being and their immune status thus resulting in reduced resistance to pathogens. The interaction between fish and microorganism is not always harmful, provided fish is not subjected to stress conditions (Udomkusonsri and Noga, 2005). Fluctuation in water temperature, pH, concentration of dissolved oxygen, crowding, handling and transportation act as a stressors and continuously effect homeostasis of fish and thus fish

become susceptible to attack by variety of pathogens. Skin infection is the most common disease in fish that may occur due to wide variety of environmental factors. Skin infection can be recognized as a pollution biomarker (Noga, 2000).

The skin of the fish unlike other vertebrates (dead keratinized) is entirely formed of living cells and covered by mucus layer. The mucus and sWkin epithelium produces many substances like antimicrobial factors, antibiotics, lysozymes, antibodies, lecithin, proteases, transferrin and carbon reactive proteins (Alexander and Ingram, 1992; Robinette *et al*., 1998). A wound on fish skin serve as superb habitat for the ubiquitous opportunistic environmental pathogens to colonize very quickly and promote osmotic stress. Bouck and Smith (1979) reported that under stress condition 10% skin ulceration leads to nearly 50% acute mortality in fish. A single fin damage can reduce 80% survival of wild fish and ectoparasites that are only restricted to epidermis of skin can kill fish under osmotic shocks due skin damage (Noga, 2000). Many studies also reports that low temperature as well as high temperature (hyperthermia) suppresses the defense strategy of fish skin. Acute hyperthermia reduces thickness of skin that may cause focal depression, apoptosis, reactive hyperplasia and necrosis (Iger *et al*., 1994). Similarly,

hypothermia (winter kill diseases) decreases the density of mucus cells thus making the fish highly susceptible for oomycete infection (Quiniou *et al.*, 1998).

Buchmann (1999) explained immune mechanisms responses of fish skin against monogeneans present over the surface of fish. Udomkusonsri and Noga (2005) reported that many species of fish are highly susceptible to the acute ulceration response (AUR). West (2006) reported that members of genus *Saprolegnia* (oomycetes) develop saprolegniosis disease in freshwater fishes characterized by development of grey or white visible patches on fins and body of fish. Thilakaratne *et al*. (2003) reported skin infections due to different parasites like monogenean trematodes, copepods crustaceans, nematodes and protozoans among 13 species of fish. An overview about the immunological defense of the fish skin was described by Esteban (2012) that mucosal immune system and mucosa-associated lymphoid tissues provide protection to fish against pathogens during first encounter. Iqbal *et al.* (2013) conducted a survey about skin infection in fishes of Trimmu Head-works area (River Chennab and Jehlum) in district Jhang, Pakistan and determined that eight species of wild fishes were suffering from various skin infections including fin rot and eye damage. Iqbal and Khatoon (2019) reported skin infection in wild commercial fishes at Head Balloki area of River Ravi. The pollution and fish kill in rivers in Pakistan have been reported. Mass mortality of *Schizothorax* sp. in River Swat (KPK) and its tributaries was reported. Industrial and domestic pollution was stated to cause fish mortality. Moreover, *Saprolegnia* sp. was also been infecting fish (Anon, 2016). The mortality of trout due to plastic pollution, bacterial and fungal infection has also been stated in River Kunhar (KPK) (Anon, 2021). Ravi is the most polluted river in the world with active pharmaceutical posing threats to environment and Human health (Gobol, 2022). The aim of present study was to calculate the extent of skin disease prevalence in commercial and non-commercial fish species of the Indus River at Taunsa Barrage, South Punjab, Pakistan. This is the first attempt to observe various type of skin infection in fish particularly in Indus River, at Taunsa Barrage, Punjab, Pakistan.

Taunsa Barrage was constructed on River Indus. Taunsa Barrage was inaugurated on 3ʳᵈ March 1959 by the then President of Pakistan, General Muhammad Ayub Khan (Late). Barrage capacity is 1,000,000 cusecs (1 million cusecs) and super flow is 1,265,000 cusecs (1.265 million cusecs). Irrigated area by Taunsa Barrage is 2.351 million acres (951, 400 ha). Three canals from Taunsa Barrage were constructed; Muzaffar Ghar canal and Taunsa Punjnad link (TP Link Canal) (from left flank) and Dera Ghagi (DG) Canal (from right flank). The fishing

rights of Taunsa barrage was leased out for last four years (2019-20; 2020-21, 2021-22, 2022-23) were 4.2; 6.6; 10.002 and 10.31Million Pak. rupees, respectively (Johar and Khan 2023a). Fish production of Taunsa Barrage for last three years (2019-20, 2020-21, 2021-22) was reported to be 20,000; 24,000, 28,000 Kg, respectively (Johar and Khan, 2023b).

MATERIALS AND METHODS

A total of 2250 specimens belonging to 70 different species were collected from Indus River at Taunsa Barrage, Punjab, Pakistan. Fish samples were collected from September 2013 to August 2014 for the period of one year randomly from eight sites from study area. Different types of nets were used for the collection of fish samples from the study area like; gill nets, drag nets, cast nets and hand nets. All the fish specimens were initially preserved in preservatives like 10% formalin and 85% ethanol packed in plastic buckets and transported to the Fish Disease and Health Management Lab, University of the Punjab, Lahore. Larger specimens were also given 10% formalin injection in their peritoneal cavity at their site of collection. Initially whole-body surface of every specimen including fins, eyes, mouth and gills were carefully observed through naked eye for the presence of any type of skin lesion or erosion on the body surface. A total of 1595 specimens belonging to thirty-five species of freshwater fish were suspected to be suffering by various types of skin infections. The fish were identified on the basis of morphometric characters by using standard key (Mirza and Sandhu, 2007). The photographs of fishes with skin infection were taken by digital camera (Sony DSC-S90, Canada).

RESULTS

A total of 1595 (about 71%) fish specimens, out of 2250 sampled fish were found to be suffering from various skin infections. The infected fish comprised 35 different species. The prevalence of skin infections was high in the commercial fishes (44%) as compared to non-commercial fishes (27%). Among the commercially important fishes the population of *Systomus sarana* (41%) was highly affected by skin infections followed by *Mastacembelus armatus* (37.5%) and *Wallago attu* (33%) while least affected fish species were *Oreochromis niloticus* (2.9%) followed by *Ompok pabda* (3.1%) and *Cirrhinus reba* (5.5%) (Table I). The highest prevalence of skin diseases was studied in *Colisa lalia* (25%), followed by *Xenotodon cancila* (20%) and *Puntius chola* (10.5%) and the lowest prevalence were recorded in *Gudusia chapra* (1.5%), followed by *Notopterus notopterus* (2.1%) and *Mystus cavasius* (2.8%)

in non-commercial fish species (Table II). The highest rate of skin diseases was recorded in November (49%), followed by October (27.9%) and February (21.3%) in the commercial fish (Table I). While highest rate of skin diseases were studied in March (30%), January (20%) and December (19.3%), respectively in the non-commercial fishes (Table II).

A total of 17 (16.2%) out of 105 *Cirrhinus mrigala* species were found to be suffering from skin infections. Left eye of one specimen was found to be surrounded with a big round circular blotch (Fig. 1A). Caudal fin of another specimen was affected with tail rot disease leaving only fin rays and rest of the fin has been damaged. Rests of the specimens were found to be having various sizes of wounds on their bodies and scales were removed (Fig. 1B).
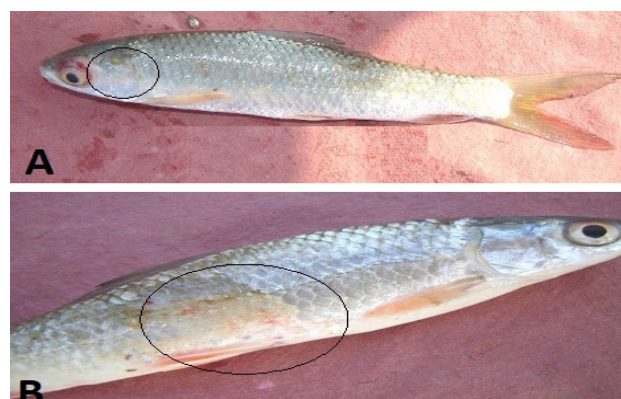
Fig. 1. *Cirrhinus mrigala* with big reddish wound surrounded whole eye (A), and with a superficial wound on the body (B).

Table I. Disease prevalence in commercial fishes of the Indus River at Taunsa Barrage.

S. No	Family/Species	Sep D/T	OC D/T	Nov D/T	Dec D/T	Jan D/T	Feb D/T	Mar D/T	Total diseased	Total healthy	Diseased percentage
I	**Cyprinidae**										
1	*Cirhinus mrigala*	0/7	--	¾	4/30	10/40	--	--	17	105	16.2%
2	*Cirhinus reba*	½	--	--	--	1/27	--	7/55	9	163	5.5%
3	*Catla catla*	--	1/2	--	--	--	2/5	--	3	19	15.8%
4	*Labeo rohita*	--	02/7	--	--	7/45	--	1/6	10	124	8%
5	*Labeo gonius*	--	--	--	--	--	1/34	5/31	6	97	6.2%
6	*Labeo calbasu*	--	--	--	--	2/4	--	--	2	11	18%
7	*Labeo dyocheilus*	--	--	--	2/6	--	--	--	2	15	13%
8	*Labeo boggut*			--	--	--	--	1/5	1	10	10%
9	*Ctenopharangodon idella*	1/1	--	--	--	--	--	--	1	6	16.7%
10	*Systomus sarana*	--	3/11	14/14	2/3	2/13	--	--	21	51	41%
11	*Securicula gora*	--	--	--	--	--	--	2	2	11	18%
II	**Cichlidae**										
12	*Oreochromis mossambicus*	--	2/5	6/26	--	1/13	--	--	9	95	9.5%
13	*Oreochromis niloticus*	--	1/5	--	--	--	--	--	1	34	2.9%
14	*Oreochromis aureus*	--	3/6	1/6	2/9	--	--	--	6	42	14.2%
III	**Channidae**										
15	*Channa punctata*	--	4/21	--	2/12	--	--	--	6	106	5.7%
16	*Channa striata*	--	1/3	--		--	--	--	1	11	9%
17	*Channa marulius*	--		--	--	--	½	--	1	12	8.3%
IV	**Mastacembelidae**										
18	*Mastacembelus armatus*	--	--	--	--	--	3/3	--	3	8	37.5%
V	**Siluridae**										
19	*Wallago attu*	--	1/3	--	1/7	1/7	2/2		5	15	33%
20	*Ompok bimaculatus*	--	--	--	--	--	--	2/3	2	7	28.6%
21	*Ompok pabda*	--	--	1/1	--	--	--	--	1	32	3.1%
VI	**Schilbeidae**										
22	Clupisoma garua	--	1/5	--	--	--	1/1	--	2	16	12.5%
	Total Diseased	2	19	25	13	24	10	18	111	990	11.21%
	Total examined	10	68	51	67	149	47	102	494	--	--
	Diseased %	20%	27.9%	49%	19.4%	16.1%	21.3%	17.6%	22.47 %	--	--

Table II. Disease prevalence in non-commercial fishes of Indus River at Taunsa barrage.

S. No	Family/ Species	Sep D/T	Oc D/T	Nov D/T	Dec D/T	Jan D/T	Feb D/T	Mar D/T	Toatal diseased	Total healthy	Diseased percentage
I	**Notopteridae**										
1	*Notopterus notopterus*	--	--	--	1/6	--	--	--	1	47	2.1%
II	**Clupeidae**										
2	*Gudusia chapra*	--	--	--	¼	--	--	1/14	2	137	1.5%
III	**Cyprinidae**										
3	*Puntius sophore*	--	--	1/56	6/19	--	--	--	7	133	5.3%
4	*Puntius terio*	--	--	--	--	--	--	½	1	16	6.2%
5	*Puntius chola*	--	--	--	--	--	--	4/12	4	38	10.5%
6	*Aspidoparia morar*	--	--	1/15	--	--	--	--	1	20	5%
7	*Salmophasia bacaila*	--	--	--	--	--	--	2	2	23	8.7%
8	*Osteobrama cotio*	--	--	--	--	--	--	½	1	32	3.1%
IV	**Bagridae**										
9	*Mystus bleekri*	--	--	--	1/12	--	1/7	--	2	66	3%
10	*Mystus cavasius*	--	--	1/38	1/5	--	--	--	2	71	2.8%
11	*Mystus horai jayram*	--	--		1/12	--	--	--	1	13	7.7%
V	**Osphronemidae**										
12	*Colisa lalia*	--	--	--	¼	--	--	--	1	4	25%
VI	**Belonidae**										
13	*Xenetodon cancila*	--	--	--	--	1/5	--	--	1	5	20%
	Total diseased	--		3	12	1	1	9	26	605	4.30%
	Total examined	--	--	108	62	5	7	30	212	--	--
	Diseased %	--	--	2.8%	19.3%	20%	14.3%	30%	12.3%	--	--

Fig. 2. A *Catla catla* with a superficial lesion at the base of dorsal fin.

Nine *Cirrhinus reba* specimens (5.5%) out of 163 were found to be affected with skin infection. A *Lernaea* sp. was observed attached on the body of one *C. reba*. Three specimens (15.8%) out of 19 *Catla catla* had skin infection (Fig. 2).

Ten specimens (8%) out of 124 collected specimens of *Labeo rohita* were affected. Six specimens (6.2%) out of total 97 individuals of *Labeo gonius* were affected by skin infection. Eighteen percent population of *Labeo calbasu* suffered by various skin infections. Almost entire trunk on both sides of one individual of *L. calbasu* was seriously affected having a large reddish wound the scales are removed and dermis is exposed (Fig. 3A, B, C).
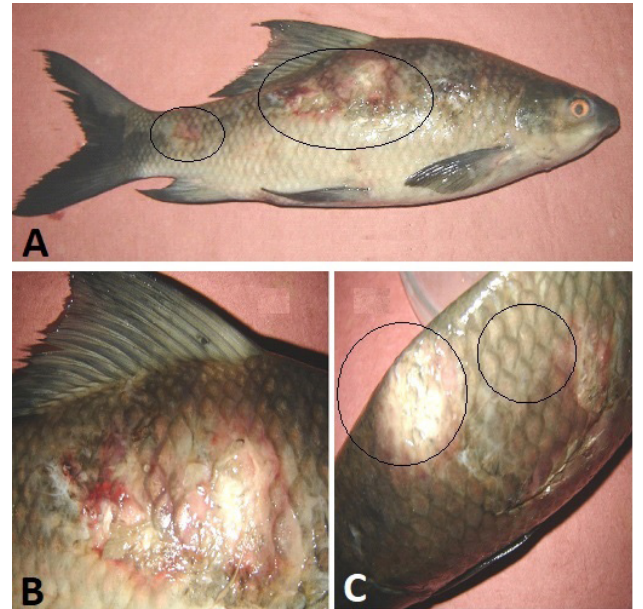
Fig. 3. *Labeo calbasu* with deep lesion below dorsal fin (scales and epidermis is eroded and musculature is exposed) and near caudal peduncle (A). B and C are magnified view of Fig-3A.
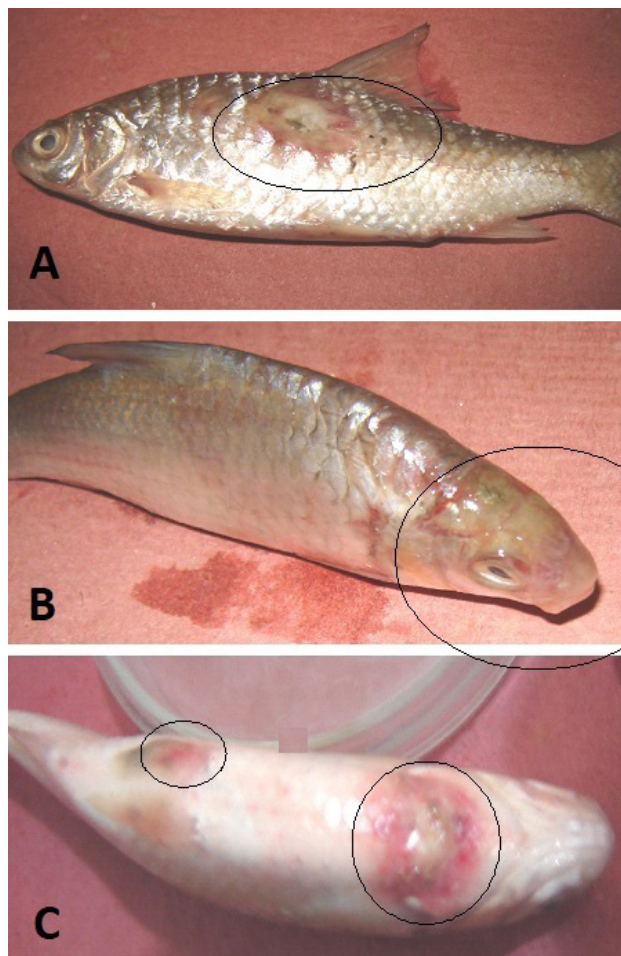
Fig. 4. S*ystomus sarana* with severely wounded trunk (A). Entire reddish head, skull and mouth (B), and severe wound on ventral side and base of pelvic fin (C).

Fig. 5. *Channa punctata* with damaged eye and lesion around middle of dorsal fin (A) and with whitish and multiple reddish wounds on middle of the body (B).
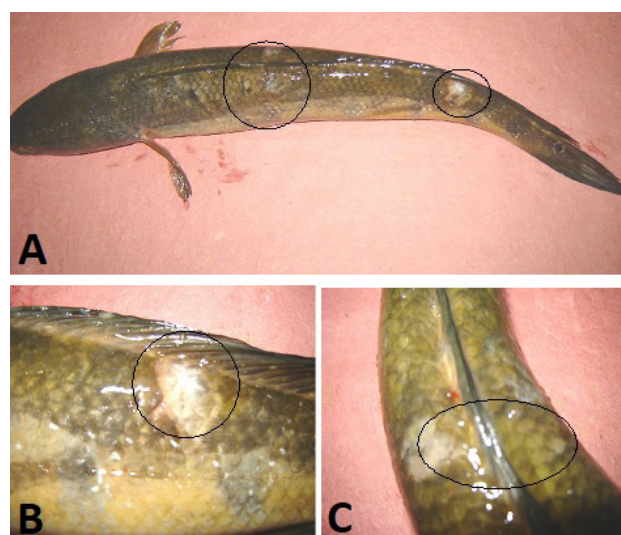

Fig. 6. *Channa marulius* with multiple wounds on body (A), B and C show magnified view of wounded trunk and base of dorsal fin.

A total of 21 (41%) out of 51 collected specimens of *Systomus sarana* were highly affected due to various type of skin infections (Fig. 4A, B, C). One specimen was found to have serious fin rot disease.

The individuals of *Oreochromis moosambicus, O. niloticus* and *O. aureus* were found suffering with mild skin and fin infections. The individuals of *O. niloticus* species were least affected (2.9%).

The overall intensity of skin infections in *Channa punctata, Channa striata* and *Channa marulius* was mild in the study area. But one specimen of *C. punctata* species was very severely affected having right eye damaged with large whitish patch. A big whitish blotch with eroded scales was present on right side of tail up to the base of caudal fin and gills were moderately damaged with bleeding (Fig. 5A, B). One specimen of *C. marulius* was moderately wounded (Fig. 6A, B, C).

A total of three specimens (37.5%) out of eight *Mastacembelus armatus* species suffered with skin infections. Two specimens were seriously affected, one having tail rot disease with completely damaged caudal fin up to caudal peduncle (Fig. 7A) and other has completely left eye damaged and large whitish red circular deep wound in the middle of body (Fig. 7B, C, D). The specimen of *Wallago attu* (Fig. 8), *Ompok bimaculatus, O. pabda* and *Clupisoma garua* species were affected with multiple wounds on their body surface which comprised 33%, 28.6%, 3.1% and 12.5% of skin infection, respectively (Table I).
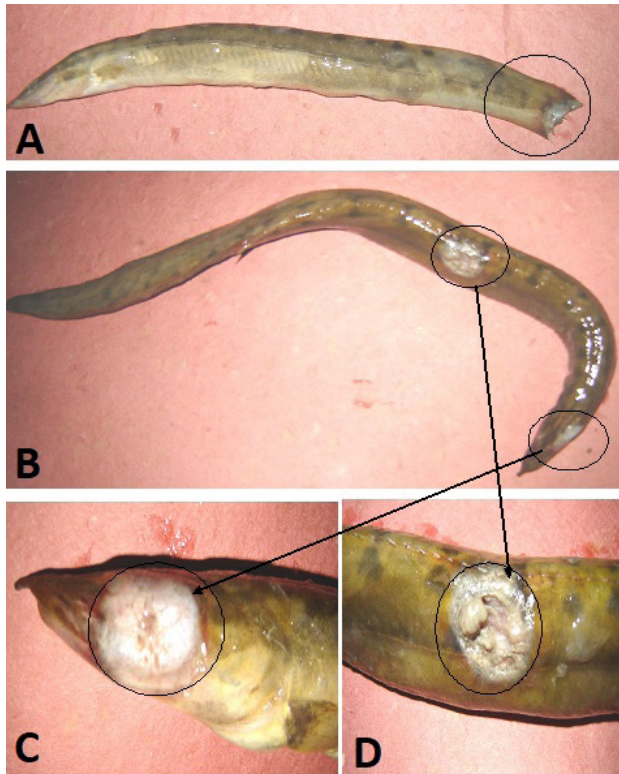
Fig. 7. *Mastacembelus armatus* with entire caudal fin damaged (tail rot) (A), damaged eye and a deep wound in center of the body (B), C and D show magnified view of complete damaged eye; large deep wound in middle of the of the body epidermis is damaged and dermis is exposed.

Fig. 8. *Wallago attu* with a large wound at the base of dorsal fin.

Among non-commercial fish species *Colisa lalia* (25%) specimens were highly affected by skin infection. The specimens of *Puntius sophore* (5.3%) were severely affected with skin infection with large reddish wound on middle of the body spreading to major portion of their trunk (Fig. 9A, B). The specimens of *P. terio, P. chola, Aspidoparia morar, Salmophasia bacaila* and *Osteobrama cotio* also suffered by skin diseases with prevalence of 6.2%, 10.5%, 5%, 8.7% and 3.1%, respectively (Table II). One specimen of *Mystus bleekri* has large reddish wound

with damaged skin in the form of large reddish papule at the front of the head (Fig. 10). The specimens of *M. cavasius, M. horai jayram, Colisa lalia* and *Xenotodon cancila* also suffered with skin infections with prevalence 2.8%, 7.7%, 25% and 20%, respectively (Table II).
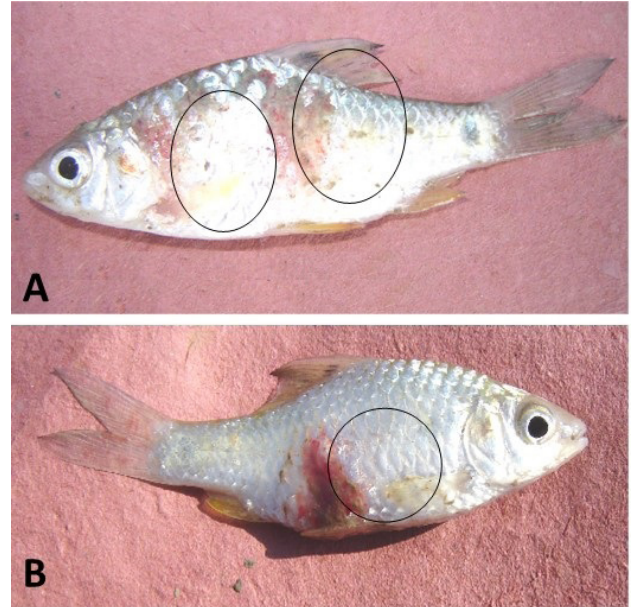
Fig. 9. *Puntius sophore* with multiple wounds on almost entire body (A) and large deep wound in middle of the body (B).

Fig. 10. *Mystus bleekri* with large reddish wound on right side behind the head.

DISCUSSION

In the present study, not a single specimen of infected fish was captured from main bed of Indus River from Taunsa Barrage. The diseased specimens were only captured from the huge lakes adjacent to Taunsa Barrage area in winter season. These lakes receive water from Indus River. There is no water connection between Indus River and these associated lakes from November

to April annually as the water level drops in the River. The prevalence of skin diseases in the study area may be associated with many factors and fish harvesting practices by contractors at Taunsa Barrage. The fish contractors repeatedly harvest fish from these lakes on large scale in winter. For harvesting fish, the contractors use various types of equipments. These equipments (nets etc.) are also extensively used in the fish farms in the surrounding areas. Hence, the chances of transmission for pathogens from private fish farms to lakes increases. The other worse and alarming situation at Taunsa barrage is that fish contractors use different types of poisons and inject electric current in water to economize expenses and to obtain huge catch from these lakes in winter season. The poison kills almost every type of living organisms in these lakes, while the surviving organisms suffer from various skin infections. Repeated and extensive fish harvesting also disturb fish continuously which lead to stressful condition for fish on one hand and on the other hand the fish which escape netting get injured. This may lead to development of skin infection in the fishes of Taunsa Barrage. Other factors that may be involved in development of skin infection included the continuous decrease in water level in these lakes. Even some time the fisherman breach the banks of these lakes or put out their water back into River by using water pumps. In these conditions aquatic life becomes congested in small volume of water and fishes become under threat of extensive and repeated attack by predators. This results in injuries and even death of fish too.

On the other hand, decrease in air and water temperature, drop in water level, stagnant water condition due to non availability of water from main River flow offer conducive habitat for various fish pathogens to multiply rapidly in such conditions. All these factors seems to be responsible for the development of skin diseases in fish species of Indus River at Taunsa Barrage. Interestingly, no influx of industrial, municipal or any other pollution from any source was observed to contaminate the Indus River at Taunsa Barrage. There is no big city or industry found to exist around the Taunsa Barrage. The city of Kot Adu is located at the distance of 15 km in the East of Taunsa Barrage and sewage water of the city is not permitted to be discharged into the River at any point. Hence, no pollution may be associated with the cause of skin infections in the fishes at Taunsa Barrage area.

Furthermore, Taunsa Barrage is located between the Chashma Barrage and Punjnad Headwork of Indus River. Chashma Barrage is located about 258 km upstream of Taunsa Barrage (where Chashma Nuclear Power plant is constructed), while Panjnad Headwork is located 202 km downstream where other four major Rivers (Chenab, Jhelum, Ravi, Beas and Satluj) of Pakistan confluence with Indus River. The water of Chenab is polluted because River Ravi and Satluj join Chenab before their confluence to Indus River at Panjnad Headwork. They receive polluted water from many cities and industries. But the source of upstream and downstream pollution might be involved very rarely in causing skin infections in the fishes of Taunsa Barrage of Indus River. This is the first report of its type from Taunsa Barrage River Indus. However, two similar studies by Iqbal *et al*. (2013) and Iqbal and Khatoon (2019) were carried out from River Chennab and River Ravi areas, respectively.

CONCLUSION

Further detailed survey should be conducted to investigate and understand the factors deteriorating the health status and skin infections of Indus River fish species at Taunsa Barrage. Moreover, the authorities should take urgent actions and formulate guidelines to protect the diverse and rich fish fauna of Indus River and its adjacent area from any future health issues.

IRB approval
The Departmental Doctoral Program Committee, of Zoology Department, University of the Punjab, Lahore approved this study.

Ethical statement
The guidelines of Ethical Committee of University of the Punjab, Lahore were followed during this study.

Statement of conflict of interest
The authors have declared no conflict of interest.

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Climate Change and Mangroves: A Potential Source of Carbon Sequestration in the Indus Deltaic Region

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ABSTRACT

This study highlights the importance of mangrove forests in the Indus Delta region of Pakistan, specifically within the administrative control of Port Qasim Authority (PQA). Mangroves are crucial for carbon sequestration and storage, and the degradation of mangrove ecosystems can threaten the livelihoods of coastal communities by destabilizing economic potential. The study found that the carbon biomass content of mangrove trees in PQA was estimated to be 33.79 tons/ha, and that there is a positive correlation between mangrove tree heights and carbon dioxide sequestration and tree diameter in well-established *Avicenna marina* trees. The study also found significant differences in tree heights observed at seven locations in PQA, highlighting the need for sustainable management and conservation efforts. The benefits of mangrove conservation include increased carbon sequestration, improved air and water quality, enhanced coastal protection, increased availability of ecosystem services, and improved land use decisions.

INTRODUCTION

The administrative control of the Port Qasim Authority (PQA) extends over 64,000 ha of mangrove forests of the Indus Delta, mud flats, and creeks, with 4,900 ha of land area above the high water mark (+3.4m. Engro Vopak Report), ESIA (2014). Industrial zones and port areas make up the primary land use in the vicinity of the site. Due to the decreased amount of nutrients available in the estuaries and creeks that connect to the sea, the growth of mangroves, which is essential to the largest single mangrove ecosystem in tropical coastal environments, is not as supported as it used to be. The Indus Delta mangrove ecosystem historically had eight mangrove species, with *Avicenna marina* being the dominant species as of today. Mangroves are essential in adjacent coastal waters, providing high biological productivity that contributes to the nutrient budget. Coastal and marine resources, including mangroves, offer numerous products and services that benefit national economic growth and development. These ecosystems support biological diversity, regulate local climate, recycle essential nutrients, absorb and break down pollutants, and mitigate the impacts of storm surges. Any significant perturbation or change in the ecological balance of established mangroves communities would destabilize species diversity and reduce the flow of energy. Coastal areas are of significant economic value. However, people living in coastal areas tend to use mangrove resources unsustainably by clearing trees for fuel and fodder, causing a reduction in coastal ecosystem productivity that can no longer support their livelihoods. Consequently, these coastal communities often migrate in large numbers to cities, increasing the pressure on the urban environment.

MATERIALS AND METHODS

Study area

The study area for carbon mangrove sequestration was located within the Port Qasim Authority, which comprises of three major creek systems: The Gharo Phitti Creek System consisting of Gharo Creek, Kadiro Creek, and Phitti Creek. The creek system spans 28 km in length and has a width ranging from 250 to 2,500 m. It is situated 22.3 km from Karachi and runs from Gharo Creek at the

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north-eastern end to Phitti Creek at the south-western end, with the Korangi and Kadiro Creeks connected to it at the north-eastern end, while it serves as the primary waterway connected to the open sea at the south-western end. The study area includes approximately 4,900 ha of land above the high water line and 64,000 ha of mangrove forests, mud flats, and creeks (Fig. 1).
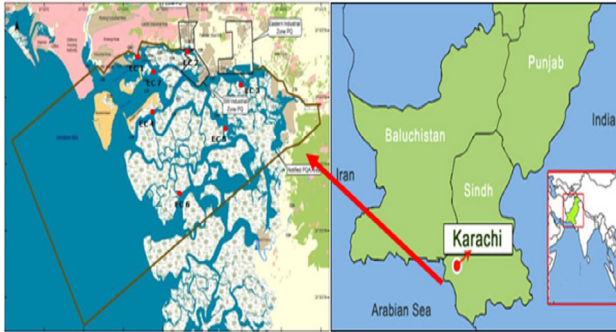

Fig. 1. Geographical location and study area, South of Karachi Port Qasim, part of the Indus Delta, Sindh.

Mangrove carbon sequestration

Mangroves are a unique tropical ecosystem that grows extensively along protected coasts with muddy to sandy bottoms covered by tidal fluxes. Mangrove ecosystems are large and dynamic reservoir of carbon, which is an important part of global carbon cycle and a potential sink of atmospheric carbon dioxide (Matsui *et al*., 2010). Mangroves store large quantities of organic carbon. Very few studies have been conducted on the biomass carbon content of mangroves species *Avicenna marina* (Pandey and Pandey 2013). They differ from forest ecosystems because they receive large inputs of organic matter and energy from both land and sea, making them a large and dynamic reservoir of carbon. Carbon cycling and storage in mangrove forests have been evaluated, and some species have been studied for their biomass carbon content.

Carbon cycling and storage in mangrove forests has been evaluated by Alongi (2014). To estimate the amount of CO_2 sequestered by trees, a process is used that involves determining the total weight, dry weight, weight of carbon, weight of carbon dioxide sequestered, and weight of CO_2 sequestered per year (To determine the weight of carbon dioxide sequestered in the tree, multiply the weight of carbon in the tree by 3.67 the ratio of the molecular weights of CO_2 to C). This gives the amount of CO_2 sequestered in the tree. To determine the weight of CO_2 sequestered in the tree per year, divide the amount of CO_2 sequestered by the age of the tree Scott DeWald *et al*. (2005).

For the mangroves in the PQA study area, the carbon biomass content was estimated by measuring the tree diameter and height, and applying a biomass equation specific to mangroves. This provides an estimate of the carbon sequestration potential of the mangroves in the area. It is important to note that these are rough estimates and actual carbon sequestration rates can vary based on factors such as species, climate, and management practices.

RESULTS

Regression analysis was conducted on mangrove tree heights, diameter, and carbon sequestered as tree biomass. Results of the analysis showed that diameter and height have a significant impact on carbon dioxide sequestered per year in kg. The coefficients of diameter and height show the increase in carbon dioxide sequestered on average per year with a one cm diameter and one-foot height increase, respectively.

The dependent variable is the amount of carbon dioxide sequestered per year, while the independent variables are the height and diameter of the mangrove trees. The analysis was based on 35 observations, with five observations taken at each of the seven-sampling location. The coefficients of diameter (β) and height (λ) indicate the effect of each variable on the carbon dioxide sequestered per year in kilograms, while the t-value reflects the significance of these coefficients. The R-square value measures the goodness of fit of the model, while the P-value indicates the significance of the model. The formula for carbon dioxide sequestration in kilograms per year is given by $\alpha + \beta$ Diameter $+ \lambda$ Height $+ \mu$, where β represents the coefficient of diameter, which indicates the average increase or decrease in carbon dioxide sequestered per year in kilograms resulting from a one-centimeter increase in tree diameter. Similarly, λ represents the coefficient of height, which indicates the average increase or decrease in carbon dioxide sequestered per year in kilograms resulting from a one-foot increase in tree height. The results of the regression analysis on the relationship between carbon sequestration, tree height, and diameter in the PQA mangrove ecosystem are presented in Table I.

Based on our findings, an increase of 1 cm in diameter of mangrove trees corresponds to an average increase of approximately 0.84 kg of carbon sequestered per year, while an increase of 1 meter in mangrove height corresponds to an average increase of approximately 6.99 kg of carbon sequestered per year in the PQA study area of the Indus Delta.

Our ANOVA results show a significant difference ($p < 0.05$) in the heights of *Avicenna marina* mangrove trees observed at seven locations in PQA (Table II).

Table I. Result of regression analysis CO_2 sequestered as carbon biomass at the sampling locations in PQA (5% significance level).

Observation area	Dependent variable is carbon dioxide sequestered per year					
	Coefficient of diameter		Coefficient of height		R square	P value
	β	t-value	λ	t-value	0.92	0.00
For total area	0.84	3	6.99	5.77	0.99	0.00
EC1	1.19	4.3	6.97	3.98	0.99	0.00
EC2	0.62	4	0.45	1.58	0.99	0.00
EC3	0.52	11	0.17	2.2	0.99	0.00
EC4	2.27	1.03	2.81	0.34	0.99	0.00
EC5	1.4	11	5.78	3.1	0.99	0.00
EC6	0.68	1.4	7.58	3.8	0.95	0.00
EC7	3.97	47	4.58	29	0.99	0.00

Table II. ANOVA mangrove tree heights observations taken from 7 different locations in PQA.

	Sum of squares	df	Mean square	F	Sig.
Between groups	48.407	6	8.068	8.566	0.000
Within groups	26.372	28	0.942		
Total	74.779	34			

Figures 2 and 3 show a positive linear regression between mangrove tree heights and CO_2 sequestered and mangrove diameter and CO_2 sequestered.
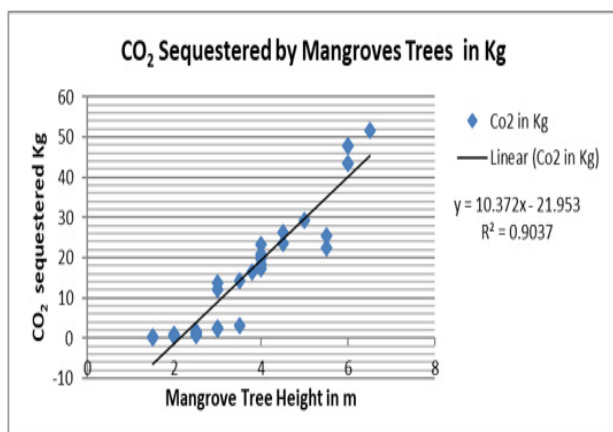
Fig. 2. Positive linear regression between mangrove tree heights and CO_2 Sequestered.
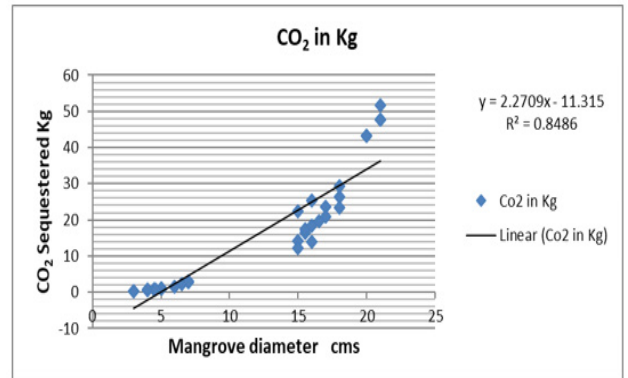
Fig. 3. Mangrove tree diameter and CO_2 sequestered. There is a positive correlation between mangrove tree heights and Carbon dioxide sequestered ($R^2 = 0.903$) and tree diameter ($R^2 = 0.848$) in well-established mangrove trees.

DISCUSSION

The study highlights the importance of mangrove conservation, replanting, and sustainable management for the local communities in the PQA area. Overexploitation of mangrove forests for wood resources has been a major issue, and this program aims to mitigate the damage caused by it. Mangroves are biologically productive and provide nutrients to the adjacent coastal waters, thereby increasing the potential for fisheries yield. The overall Productivity in the mangrove areas is reported to be high (365-780gC/m²/year, IOC, 1994), which accounts for greater potential for fisheries yield in the PQA (64,000ha) mangrove area (Amjad *et al*. 2016). They export organic matter, mainly in detritus form (leaf litter) to the marine environment, thus providing a highly nutritious food source for themselves and for animals found in the mangrove areas, as well as for those in neighboring estuarine and marine ecosystems. (Snedaker, 1984). Coastal communities benefits in a multitude of ways from Mangrove ecosystems. These ecological functions are collectively known as ecosystem services and are essential for the provisioning of food and services, decomposition of organic wastes, and supporting the growth of mangroves and aquatic life. Mangroves are a unique tropical ecosystem that grows extensively along protected coasts with muddy to sandy bottoms covered by tidal fluxes. They differ from forest ecosystems because they receive large inputs of organic matter and energy from both land and sea, making them a large and dynamic reservoir of carbon. Carbon cycling and storage in mangrove forests have been evaluated, and some species have been studied for their biomass carbon content.

Mangrove conservation efforts are crucial for carbon

sequestration and mitigating climate change in tropical coastal areas. Clearing of mangroves can lead to significantly reduced carbon stores. The study highlights the potential for carbon sequestration through rehabilitation activities such as reforestation, which can be achieved through industrial corporate social environmental responsibility (ICSR). The benefits of mangrove conservation include increased carbon dioxide sequestration, improved air and water quality, enhanced coastal protection from erosion and storm surge, increased abundance and diversity of indicator species, increased availability of mangrove ecosystem services in nearby communities, increased awareness of the value of mangrove ecosystems, more sustainable use, and improved land-use decisions.

ACKNOWLEDGEMENT

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The Study of Bacillariophyta Species Diversity in the Coastal Waters of Lasbela, Baluchistan

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ABSTRACT

The investigation of the variety of phytoplankton species in the coastal waters of Lasbela, Balochistan was conducted. Seasonal sampling was carried out from both inshore, Miani Hor Lagoon and offshore, the surrounding open sea between periods of September 2019 to August 2020. Over the course of three seasons (post monsoon, pre monsoon, and monsoon seasons), 119 phytoplankton species from inshore and offshore water were recognized as planktonic. Two types of phytoplankton were identified during the seasonal sample (Bacillariophyta, and Dinophyta). The relative abundance of division Bacillariophyta taxa across the post-monsoon, pre-monsoon, and monsoon seasons revealed significant variations in species composition. Group Bacillariophyta comprised 62% of the species in offshore water and 58% of the species in inshore water during the post-monsoon season. Division Bacillariop/hyta species made up 49% of inshore and 57% of offshore species in the pre-monsoon season. Division Bacillariophyta planktonic algae were estimated to make up 53% of species in inshore water and 55% of species in offshore water during the monsoon season. The Bacillariophyta division of planktonic algae, which consists of 25 taxa, 17 families, and 53 species, was present in inshore water. The group Bacillariophyta, observed in inshore water consists of 25 genera, 17 families, and 53 species of planktonic algae.

INTRODUCTION

Diatoms (Bacillariophyta) is one of the most varied and ecologically significant categories of phytoplankton (Bhattacharjee *et al*., 2013). They make up a significant portion of aquatic biomass, especially during prominent seasonal phytoplankton blooms, and are thought to account for up to 20% of all primary production on Earth (Uitz et al., 2010). They are widely distributed in almost all aquatic habitats, excepting the warmest and most hypersaline environments (Glaser and Karsten, 2020), and can also occur as endosymbionts in dinoflagellates and foraminifers (Kociolek and Hamsher, 2017; Holzmann *et al*., 2006).

They are thought to be especially significant in high latitudes and nutrient-rich coastal environments, but much less so in the oligotrophic open ocean (Carstensen *et al*., 2015;

Boyd *et al*., 2010). Diatoms are cosmopolitan and ubiquitous in distribution, present in freshwater and oceans (Kociolek *et al*., 2017; Malviya *et al*., 2016). Diatoms can also be found in terrestrial environments in the soil (Foets *et al*., 2020; Barragán *et al*., 2018).

According to Falkowski *et al*. (1998), between 50 and 85% of the oxygen in the Earth's atmosphere comes from phytoplankton. They are well suspended in the water column, ebbing and flowing with the currents, and have little to no control over their horizontal placement in both saltwater and freshwater settings (Fossum *et al*., 2019).

Estuaries typically have high levels of productivity and substantial phytoplankton concentrations (Cloern, 1987). As a result, phytoplankton is essential for limiting global warming and preserving the overall health of the planet's atmosphere (Simó, 2001; Finkel *et al*., 2005). Deeper layers of the water transmit nutrients to the surface, where phytoplankton flourishes, while the topmost layer of the ocean cools (Hegseth and Sundfjord, 2008). By generating currents that bring nutrient-rich deep water to the surface, winds facilitate the spread of phytoplankton (Hoegh-Guldberg and Bruno, 2010). Phytoplankton is regularly generated to enhance aquaculture, and it was crucial for controlling carbon dioxide and oxygen levels in the Earth's atmosphere prior to the Precambrian Period. Numerous studies have examined the impact of natural elements on phytoplankton dynamics (Boney, 1989; De

Huszar and Nina, 1998; Kagalou *et al*., 2008; Hassan *et al*., 2008).

In order to assess of the phytoplankton diversity assemblages in the coastal waters of Lasbela, Baluchistan this study was undertaken. Annual changes in the taxonomic composition of phytoplankton, relative quantity of phytoplankton in the sample and the comparison of the physicochemical properties of the phytoplankton at different sampling sites, were also investigated.

MATERIALS AND METHODS

Sampling site

To evaluate the phytoplankton data for seasonal fluctuations, the Miani Hor lagoon was chosen as the sampling site (inshore water 25°45.151′N, 66°55.966′E, and offshore water 25°45.539′N, 66°53.888′E) (Fig. 1).

Sample collection

Phytoplankton data were collected once a month for six months to study at seasonal variations. The pre-monsoon season lasts from January to April 2020; the monsoon season lasts from May to August 2020; and the post-monsoon season lasts from September to December 2019.

Seasonal sample collection took place from September 2019 to December 2020, with one-month intervals between each cycle. Pre-monsoon season is from (January to April 2020), Post-monsoon season is from (September to December 2019), and monsoon season is from (May to August 2020).

Samples were taken at both locations using a boat that was towing a phytoplankton net (mesh size 55 µm) horizontally across 100 m of depth beneath (0.5 metres) of water. The boat was being towed at a constant speed of 4 knots. Plankton net's filtrate was right away transferred to a 1-L bottle, where it was preserved with 5% formalin.

In order to assess Seasonal physical and chemical parameters (salinity, temperature, and pH) of the seawater from both Miani Hor sampling stations were also collected. The temperature, salinity, and pH of the water were measured in-situ using a thermometer, a refractometer, and a portable pH metre. Observation of phytoplanktons was made under the microscope (Wetzlar XLZPN).

Identification

Different sources were employed to categorise phytoplankton samples: Hoque *et al*. (1999), Chaghtai and Saifullah (1988, 2001), Richard (1987), Fritsch (1961), Prescott (1962), Kasymov (2000) and Tiffany and Britton (1971).

Quantitative estimation of phytoplankton

Total phytoplankton abundance was calculated using cells L^{-1}, and it was then reported as a percentage of total phytoplankton abundance. Each genus was tallied rather than each individual species because each genus held a sizable number of species.
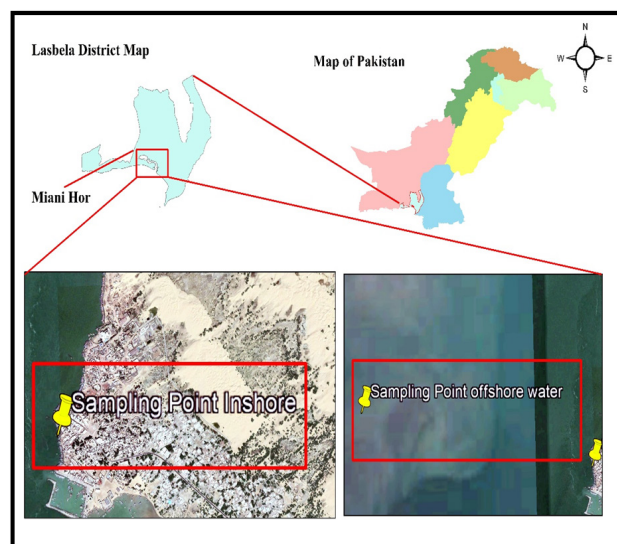

Fig. 1. A Map showing the inshore and offshore sampling sites at Miani Hor (Lagoon) Lasbela Balochistan, Pakistan.

RESULTS

Phytoplankton composition of Miani Hor, Balochistan's inshore and offshore waters, as well as its more abundant seasonal flora, are presented.

Diatoms and dinoflagellates were found in the samples from the lagoon and open sea simultaneously from September 2019 to August 2020, the study period. The diatom species were recorded from both sampling sites.

Bacillariophyta in inshore and offshore waters
  Pre-monsoon season
  Members of the phytoplankton algae group Bacillariophyta were detected in lower concentrations (44.26%) in inshore water and greater concentrations (55.76%) in offshore waters during the pre-monsoon season of the year (Fig. 2A).

  Monsoon season
  Members of the group of phytoplankton species were detected in inshore water in lower numbers (53.06%) during the monsoon season of the year and in larger numbers (46.94%) in offshore waters (Fig. 2B).
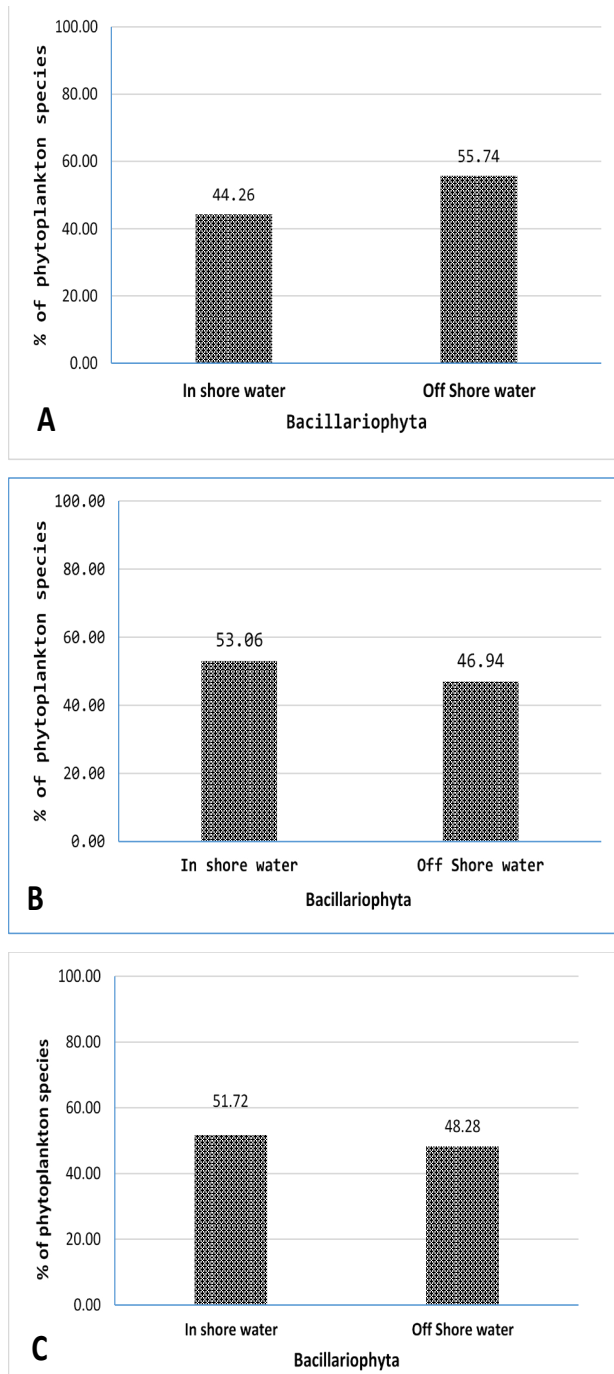
A

B

C

Fig. 2. Bacillariophyta species recorded in the inshore and offshore waters of Miani Hor, Balochistan during monsoon seasons. A, pre-monsoon; B, Monsoon; C, post-monsoon seasons.

Post-monsoon season

Members of the group Bacillariophyta were encountered in both inshore and offshore waters during the

post-monsoon season 51.72% and 48.82%, respectively (Fig. 2C).

Phytoplankton abundance

When counting the quantity of phytoplankton species, it was observed that, 2782×10^2 cells l^{-1} were counted of inshore and offshore waters in Miani Hor, Balochistan. During the period September 2019 to August 2020, Bacillariophyta (Diatom) density was 1651×10^2 cells l^{-1}.

The abundance and distribution of planktonic algae in each group during the monsoon season were also investigated. Group Bacillariophyta planktonic algae were estimated 51.12% in inshore water and 48.88% from offshore waters (Fig. 3).
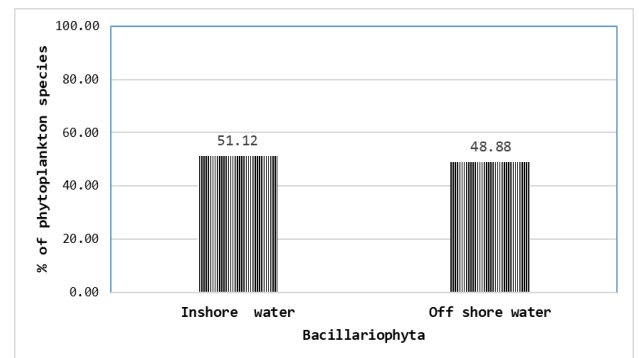
Fig. 3. The abundance of Phytoplankton species recorded in inshore and offshore waters throughout three seasons at Miani Hor Balochistan.

Seasonal variation in phytoplankton density

The seasons were divided into post monsoon (September 2019 to December 2019), pre monsoon (January 2020 to April 2020) and monsoon (May 2020 to August 2020). The data does not represent the actual picture of phytoplankton density because no collecting was feasible in June and July owing to sea roughness.

In the current study, the Bascillariophyta includes a wide range of species with various structural variations. Numerous species have been observed in very high concentrations in both inshore and offshore waters between the months of September 2019 and August 2020. *Coscinodiscus radiates, Prorocentrum micans, Thalassionema* sp., *Pseudonitzschia australis, Thalassionema nitzschioides, Chaetoceros costatum,* and *Chaetoceros armatum* are just a few of the species that have been identified as belonging to the genus *Coscinodiscus*.

Post-monsoon season at Miani Hor saw the highest growth of phytoplankton species, while pre-monsoon and monsoon seasons saw the lowest ratio. The highest ratio of *Ceratium furca* was seen in all three seasons. *Rhizosolenia*

setigera was second in terms of abundance throughout all three seasons. During the post-monsoon season, it was noted that there were 12 families and 22 genera of phytoplankton species from the group Bacillariophyta.
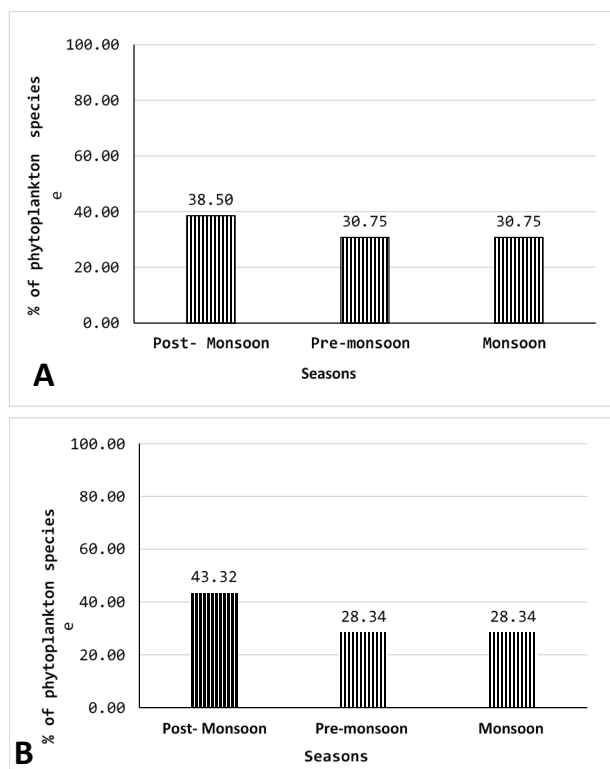

Fig. 4. Total phytoplankton species (%) from inshore waters (A), and offshore waters (B) during three seasons (post-monsoon, pre-monsoon and monsoon seasons).

There were 17 families and 24 genera of group Bacillariophyta discovered from both sites (inshore and offshore) during the pre-monsoon season. It was discovered through phytoplankton sample conducted during the monsoon season that group Bacillariophyta consisted of 15 families and 22 species (Fig. 5).

Pre-monsoon (30.75%) and monsoon (30.75%) seasons had lower ratios of group Diatoms observed than the post-monsoon (38.50%), in the inshore waters (Fig. 6).

Additionally, it was observed that in the offshore waters, Bacillariophytes were more prevalent in the post-monsoon season (43.32%), whereas species in pre-monsoon (28.34%), and monsoon seasons (28.34%), respectively (Fig. 7).

Richness and diversity of species
After counting each of the samples that were found, a statistical analysis was carried out.
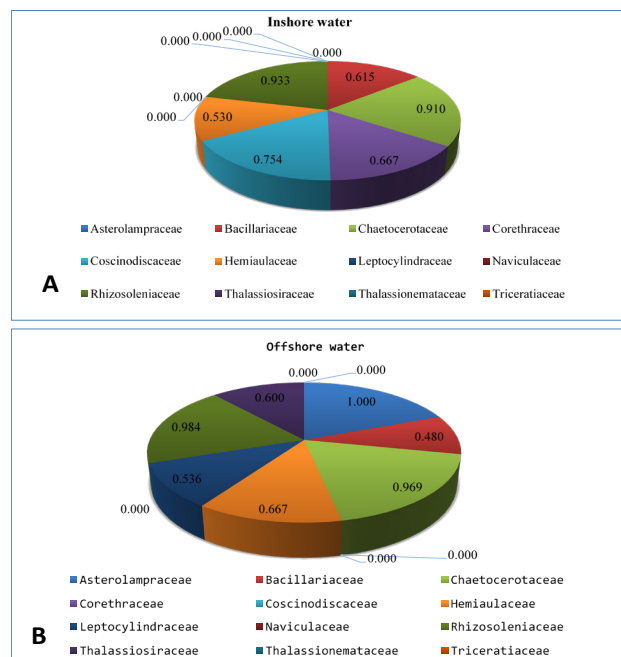
Fig. 5. Diversity index value of group Bacillariophyta throughout inshore (A) and offshore (B) waters of Miani Hor Balochistan during post-monsoon season.
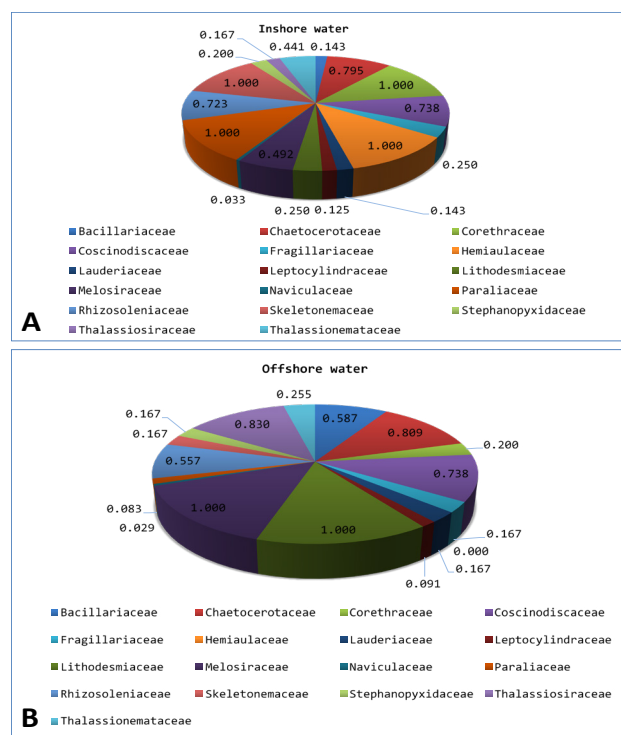
Fig. 6. Diversity index value of Group Bacillariophyta throughout the inshore (A) and offshore (B) waters at Miani Hor, Balochistan during pre-monsoon season.
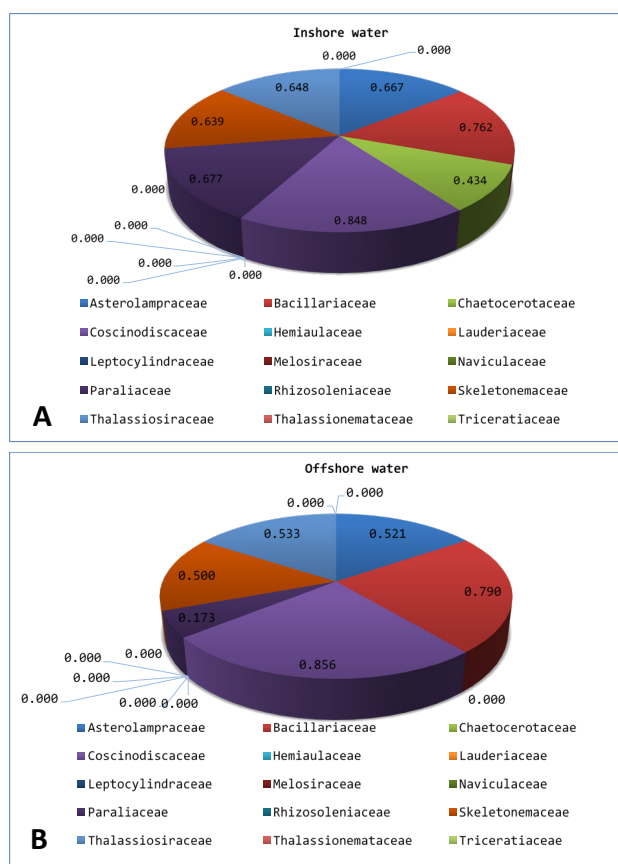
Fig. 7. Diversity index value of Group Bacillariophyta throughout the inshore (A) and offshore (B) waters of Miani Hor Balochistan during monsoon season.

The diversity index was calculated using the Simpson diversity index equation:

$$D = \Sigma\ (n-1)/(N-1)$$

Index Diversity is the initials D. The number n represents all members of a certain species of organism. N is the total number of organisms in a family, across all species.

According to the diversity index, an organism or family has very little diversity if it is found in the range between zero and one, and it has extremely large diversity if it is found in the range between one and two.

Post-monsoon season

Throughout the post-monsoon season, the results caused the diversity index value to range from 0.420 to 0.933 in inshore waters and from 0.389 to 1.00 in offshore waters.

Twelve groups of Bacillariophyta were found in both inshore and offshore seas during the Post-monsoon Season. The Group Bacillariophyta family Asterolampraceae has

the maximum diversity in D=1.00 offshore water but the lowest diversity in D=0.000 inshore water. With D= 0.933 diversity in inshore water and D= 0.984 varieties in offshore water, Rhizosoleniaceae was the second most diverse family. Three families (Naviculaceae, Thalassionemataceae, and Triceratiaceae) demonstrated very low diversity during the post-season, with D=0.000 in figures across all families (Fig. 5).

Pre-monsoon season

The results show that the diversity index value varied throughout the pre-monsoon season from D= 0.033 to D= 1.00 in inshore waters and from D= 0.029 to D= 1.00 in offshore waters.

Figure 6 shows that 17 Bacillariophyta families were found in both inshore and offshore waters during the pre-monsoon season. The three Bacillariophyta families with the highest diversity values in inshore water are Corethraceae, Hemiaulaceae, and Paraliaceae. And with D= 0.250 to D= 1.000 diversity values in offshore water, the families Lithodesmiaceae and Melosiraceae were the most divergent families. The Naviculaceae family displayed the least variety over the course of the season, with D= 0.033 figures in inshore water and D= 0.029 figures in offshore water (Fig. 6).

Monsoon season

The results show that throughout the monsoon season, the diversity index value varied from D= 0.420 to D=1.0 in inshore waters and from D=0.173 to D=0.856 in offshore waters.

15 Bacillariophyta groups were found in both inshore and offshore waters during the monsoon season. The family Coscinodiscaceae exhibited the greatest variation in Inshore water D=0.848 and Offshore water D=0.856 out of all the families in the Group Bacillariophyta. Eight families, with D=0.000 in figures, showed very little diversification throughout the monsoon season: Hemiaulaceae, Lauderiaceae, Leptocylindraceae, Melossiraceae, Naviculaceae, Rhizosoleniaceae, Thalassionemataceae, and Triceratiaceae (Fig. 7).

Pennate and centric diatom

When samples of inshore and offshore water were assessed, it was evident that centric diatoms made up 27.45% of the sample whereas pennate diatoms made up 75.55% (Fig. 8).

The relationship between phytoplankton and environmental variables.

There was no significant relationship between the total number of phytoplankton and salinity (r = -0.993; p

= 0.073), temperature (r = -0.987; p = 0.104), or pH (r = 0.721; p = 0.488) in inshore water. Similar results were seen for total phytoplankton in offshore water, where there was no statistically significant relationship between total phytoplankton and salinity (r = -0.886; P = 0.307), temperature (r = -0.862; P = 0.338), or pH (r = 0.423; P = 0.722).

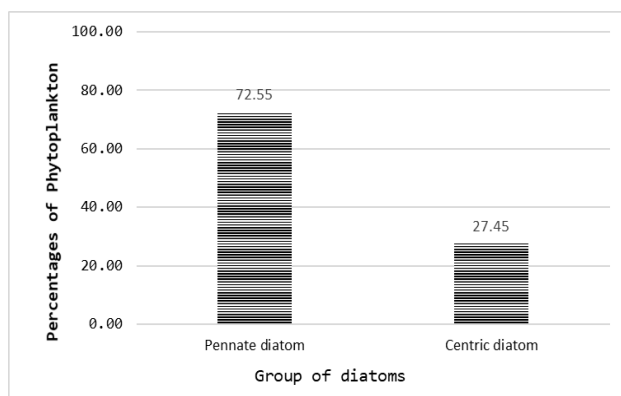Fig. 8. Showing the proportion of Pennate and Centric Diatoms distributed in inshore and offshore water during three seasons (post-monsoon, pre-monsoon, and monsoon season) at Miani Hor, Lasbela Balochistan.

DISCUSSION

The current study provides information on the distribution and abundance of key diatom genera in connection to environmental conditions. The phytoplankton abundance appears to change with the seasons, with a unimodal distribution peak occurring during the northeast monsoon between October and February. This could be as a result of the high upwelling that frequently occurs in the central and northern Arabian Sea, which increases primary production and regulates the distribution and abundance of phytoplankton in the area (Khokhar *et al.*, 2016). High productivity in the northern Arabian Sea during the northeast (winter) monsoon period is caused by wind-induced convective mixing and up-sloping of nutrient-rich water, even though upwelling along the Pakistani coast is unknown (Banse, 1968; Schapira *et al.*, 2008).

The high primary productivity of coastal waters is also influenced by the input of dissolved nutrients through rivers (Cebrián and Valiela, 1999). The study area along the coastal belt receives discharges from the Porali and Windor seasonal run-off rivers (Saifullah and Rasool, 2002).

In the lagoon and nearby open sea at the Miani Hor, 63 species of diatoms and 23 species of dinoflagellates were collected (Latif *et al.*, 2013).

Coscinodiscus, Chaetoceros, Eucampia, Rhizosolenia, and *Thalassionema* species were discovered in the Manora Channel during the survey period, according to a previous study (Shahnaz *et al.*, 2018). In the current study, these species were also seen in Miani Hor, Balochistan.

The current data would be used as a starting point for future research in the area because there is little information currently available about the phytoplankton composition and abundance in the Maini Hor Lagoon.

Phytoplankton species were the most abundant (46%) during the pre-monsoon season. It was 31% and 23% during the post-monsoon and monsoon seasons, respectively. Solak *et al.* (2012) shown that species richness was higher in winter than in summer while studying diatoms in Felent Creek, Turkey.

While pre-monsoon and monsoon seasons had the lowest number of species richness, post-monsoon had the highest number of species.

Global warming and sea level rise appear to have a significant impact on the Arabian Sea, which changes seasonality, intensifies the monsoon seasons, and boosts productivity (Goes *et al.*, 2005).

Since sea level is generally rising due to global warming, many physical factors, including precipitation, inland runoff (volume of fresh water), climatic seasonality, sea surface temperature, acidification, etc., have an impact on the parameters that determine the vertical water column (Riebesell, 2004; Moore *et al.*, 2008).

The seasonal data on the spatio-temporal distribution of diatoms in Pakistani coastal waters reported here demonstrate seasonal variability in diatom abundance and distribution and urge further study to monitor the impact of rapidly changing climatic influences that promote growth of bloom-forming planktonic species in the Arabian Sea.

Statement of conflict of interest
The authors have declared no conflict of interest.

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Exploring Awareness about Global Warming Among Prospective Teachers

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ABSTRACT

Global warming is the recent and ongoing raise in earth surface temperature. This study was designed to explore the awareness about global warming among prospective teachers at the University of Punjab. Total 500 hundred students were examined using a questionnaire about global warming. The result showed that students have average awareness about global warming. We used chi square to find out the difference of awareness level of prospective teachers about global warming with respect to different demographic variable. It shows that there are significant differences with respect to demographic variable (age, gender, department, program, CGPA, shift, and residential status) among prospective teachers. Therefore this study recommended integrated environmental concepts into the university curriculum for all students regardless of their academic specialization in order to increase the environmental awareness.

INTRODUCTION

Global warming has become an increasingly prominent topic of research and discussion over the past few decades. Rising temperatures worldwide and a higher frequency of natural disasters have drawn significant attention to this issue. The potential impacts of global warming on the future of the global population, particularly in coastal areas and regions prone to natural disasters, have made it a subject of great importance.

Global warming refers to a specific period of the larger Earth's climate system that typically involves a temperature increase. Scientists have identified several factors that can contribute to the changing climate on Earth over extended periods. These factors include solar radiation levels, the Earth's orbit around the sun, volcanic activity, ocean currents, and even plate tectonics. The periods of warming and cooling are referred to as interglacial and glacial periods,

respectively, with the latter characterized by the presence of large ice sheets extending from the poles (Singer and Avery, 2007)

Climate change is commonly associated with events such as hurricanes, but these events are not considered direct evidence of imminent global warming or a "hot crisis" (Ungar, 2000). Research on "mental models" has shown that many individuals in the United States believe that the reduction of greenhouse gas (GHG) emissions can be postponed until further evidence is presented to demonstrate their detrimental effects on climate change (Sterman and Sweeney, 2007). Despite increased exposure to the consequences of global warming, people may not react strongly due to the abstract and statistical manner in which global warming and its impacts are often portrayed (Weber, 2006).

Concerns about global warming began to emerge in the 1970s, but it was the publication of the Brundtland Report, "Our Common Future," in 1987 that garnered public attention on climate change for the first time. The report addressed various environmental problems, including climate change, and emphasized the warming trend in the world's climate resulting from unsustainable human development practices (UNWCED, 1987).

Since 2010, there has been a noticeable increase in the number of articles published on the topic of global warming. This increase reflects the importance of understanding and documenting this phenomenon to

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facilitate action, prevention, and preparation for the future. Further research can help identify gaps in the literature and shed light on the measures necessary to raise awareness of global warming among prospective teachers.

Global warming is commonly discussed as a significant global impact affecting various parts of the world. It is defined as the elevation in the temperature of the Earth's and ocean's surfaces due to the increased presence of greenhouse gases in the atmosphere (Çimer *et al*., 2011).

Global warming is emerging as a major threat to the Earth in the 21st century. It not only has adverse effects on human health but can also trigger natural disasters such as floods, famines, droughts, and cyclones (Ramzan, 2021). The Intergovernmental Panel on Climate Change (IPCC) has reported that the industrial revolution of the mid-19th century has already caused a 1°C increase in global temperature, and projections suggest that it may rise by 1.1°C to 6.4°C over the course of the 21st century. This temperature rise, coupled with the loss of biodiversity, could have devastating effects on health.

It is crucial for students and individuals to comprehend the impact of climate change and take action to address it. This starts with understanding the causes of global warming and its effects, enabling the identification of solutions and their implementation. Therefore, it is of great importance to educate people and create awareness about global warming by incorporating relevant topics into educational curricula at all levels. It is also essential to dispel misconceptions about global warming that may arise from mass media. Environmental education on global warming, from elementary school to university level, has been recognized as the most effective approach to foster environmental consciousness among nations (Anderson and Wallin, 2000; Skamp *et al*., 2009; Taber and Taylor, 2009; Kilinc *et al*., 2011).

Currently, there is a lack of available studies on the awareness of students regarding environmental issues, including global warming, in Pakistan. However, numerous international studies have been conducted on students' perceptions, ideas, and understanding of the greenhouse effect, climate change, and global warming (Bozdogan, 2009; Liarakou *et al*., 2011; Hasiloglu *et al*., 2011; Yazadanparast *et al*., 2012).

Most of the existing literature in this area focuses on students' perceptions of global warming, ranging from kindergarten-age children to high school students. Some studies also examine the role of teachers and formal environmental education in raising awareness (Boyes and Stanisstreet, 2001; Bozdogan, 2009; Yurttas and Sulun, 2010; Cimer *et al*., 2011; Ozbayrak *et al*., 2011; Owolabi *et al*., 2012).

Various international treaties have been signed to reduce greenhouse gas (GHG) concentrations in the atmosphere on a global scale. However, it is equally important to achieve reductions at the national level to fulfill this universal goal, and raising awareness is one of the initial steps towards achieving that (Arslan, 2012).

The awareness of university students, particularly science students, regarding the environment, global warming, and the greenhouse effect is expected to be relatively high compared to other students in the formal education system. This awareness is also considered an important indicator of the general population's knowledge (AbuQamar *et al*., 2015). However, research has shown that there are no significant differences in awareness levels between students who are newly admitted to the university and those at higher academic levels. This suggests that students' knowledge in this area is primarily acquired through general education rather than specific academic achievements at the university level (AbuQamar *et al*., 2015).

The aim of this study is to assess the level of awareness of global warming among prospective teachers. Since these individuals will play a crucial role in educating and shaping the minds of future generations, it is important to understand their knowledge and understanding of global warming and climate change. The study seeks to determine how well-equipped these prospective teachers are in teaching effective strategies for protecting the Earth from the impacts of global warming. By assessing their awareness, the study aims to identify any potential gaps or areas that require further attention in their training and education. Ultimately, this research will contribute to improving environmental education and empowering future teachers to effectively address global warming and climate change in their classrooms.

MATERIALS AND METHODS

The instrument

In this study questionnaire as research instrument was used for data collection. Environmental Issue Questionnaire that was developed by Boyes *et al*. (1993) and Liarakou *et al*. (2011) was adapted. The instrument consists of 30 close-ended statements. The questionnaire consisted of two parts. First part focused on demographic factors such as (gender age, department, program, semester, CGPA, and shift and residence status). Second part is consisted of 15 items whose response were collected on true /false scale and 15 items whose responses were collected on four options scale to check current awareness of global warming among prospective teachers.

The study group

The researchers personally collected the data to as explore global warming awareness among prospective teachers. Questionnaire were distributed to the prospective teachers of I.E.R of University of the Punjab and requested to fill the questionnaire with patience and care. Researchers stayed with them and if they needed any query about any statement so that could be clear to them. After filling, questionnaires were collected from the students by thanking them for their kind consideration.

Statistical analysis

After collecting data each questionnaire was given a code. After coding, data were entered into the SPSS (Statistical Package for Social Science) version 22.0. For research question 1 artificial 3 categories were formed i.e., low (1-10), average (11-20), and excellent (21-30). Percentage and frequencies were calculated to check the level of awareness of prospective teachers about global warming. Chi square test was applied it to find out the difference of awareness level of prospective teachers about global warming with respect to different demographic variables.

RESULTS

The purpose of this research was to explore the awareness of prospective teachers. Results of the research indicate that the prospective teachers have average awareness about global warming (Table I) only 4.5 of the prospective teachers had excellent awareness of global warming while 12.5 showed very low level of awareness.

Table I: Frequency and percentage prospective teachers' awareness about global warming.

Sr. #	Awareness level	Frequency	Percentage
1	Average	440	83.0%
2	Excellent	24	4.5%
3	Low	66	12.5%

It has been identified that prospective teachers with respect to residential status have negligible significant difference in the awareness about global warming. Male and female prospective teachers slightly differ in their awareness about global warming. Prospective teachers having different CGPA slightly differ in the awareness about global warming. Prospective teachers of different ages have adequate difference in the awareness about global warming (Table II).

Table II: Chi square test on knowledge about item and demographic variable.

Demographic variable	Average rising in sea level χ^2	α	The use of solar energy effect χ^2	α	Caron dioxide gas polar χ^2	α	Ice cover of earth's two polar χ^2	α	Displacement of human population χ^2	α	Rise in average global temperature χ^2	α	The growing incidence of skin cancer χ^2	α	Fossil fuel combustion increases atm. Temp. χ^2	α	Prefer use of public transport instead of private cars χ^2	α	The use of solar energy worsens global warming χ^2	α
Gender	0.68	0.40	1.22	0.26	0.00	0.96	0.76	0.38	3.13	0.07	0.02	0.88	0.04	0.83	0.98	0.32	0.06	0.79	0.04	0.82
Age	8.50	0.03	7.00	0.07	2.99	0.39	3.71	0.29	1.77	0.62	3.24	0.35	8.89	0.03	6.10	0.10	6.56	0.08	3.42	0.33
Department	10.74	0.15	11.02	0.13	30.39	0.00	5.34	0.61	4.80	0.68	9.82	0.19	17.09	0.01	13.54	0.06	28.07	0.00	4.00	0.78
program	15.03	0.18	13.74	0.24	30.88	0.00	10.41	0.49	12.57	0.32	12.17	0.35	18.36	0.07	17.39	0.09	38.52	0.00	7.49	0.75
CGPA	6.57	0.08	8.29	0.04	10.09	0.01	2.06	0.55	2.64	0.45	9.93	0.01	5.01	0.17	5.18	0.15	14.60	0.00	.50	0.91
Shift	6.63	0.08	0.36	0.94	0.56	0.90	1.88	0.59	2.42	0.48	4.46	0.21	1.69	0.63	2.21	0.53	1.44	0.69	2.01	0.57
Residence status	0.07	0.78	0.05	0.82	0.94	0.33	0.01	0.92	5.20	0.02	4.13	0.04	0.01	0.91	0.94	0.33	0.41	0.51	0.15	0.69

Prospective teachers of different semester have average difference in the awareness about global warming. Prospective teachers of different program have considerable significant difference in the awareness of prospective teachers about global warming. Prospective teachers of different have maximum significant difference in the awareness about global warming (Table II).

DISCUSSION

Global warming is frequently discussed as a significant impact affecting various regions worldwide. It refers to the rise in Earth's and ocean's surface temperatures caused by an increase in greenhouse gas concentrations in the atmosphere (Çimer *et al*., 2011).

However, despite increased awareness, there remains a notable percentage of the global population that is still unaware of climate change. Many individuals only associate climate change with variations in weather patterns, and there is a lack of understanding in distinguishing between climate change and global warming. Therefore, it is crucial to raise awareness about climate change, particularly through education, as studies have emphasized (Bostrom *et al*., 1994; Christianson, 1999; Pugliese and Ray, 2009).

The results of present study show that mostly students have average awareness about global warming but on the basis of demographic variable it varies it prospective teachers' shows significant difference in the awareness about global warming. Significant difference in demographic variables are as follow:

It has been identified that prospective teachers with respect to residential status have negligible significant difference in the awareness about global warming. Male and female prospective teachers slightly differ in their awareness about global warming. Prospective teachers having different CGPA slightly differ in the awareness about global warming. Prospective teachers of different ages have adequate difference in the awareness about global warming.

Prospective teachers of different semester have average difference in the awareness about global warming. Prospective teachers of different program have considerable significant difference in the awareness of prospective teachers about global warming. Prospective teachers of different have maximum significant difference in the awareness about global warming.

Pakistan has been ranked among the top ten most affected countries because of global warming in the past twenty years and expected to have wide-ranging impacts. Agricultural yield, availability of water could be greatly affected and increased coastal erosion, sea water incursion and increased extreme climatic events could occur more frequently (Ramzan, 2021). To control the global warming we need to educate our students .

As in majority of the countries, in Pakistan also film media is most widely used media for entertainment. This media can be used for the purpose of creating waves of awareness about the global warming, its hazardous effects on mankind, as well as the preventive measures. Pakistan film industry must step forward and contribute to combat against global warming by sensitizing general population.

CONCLUSION

Global warming is continuous increase in earth atmosphere, higher the temperature worsen it increases the disasters which include storms, floods, heat wave, and droughts. So its awareness is necessary to control the global warming and climate changes. This research is used to see how much awareness our future teachers have the result show that they have average awareness. Our prospective teachers must aware of global warming consequence so they can teach to future students .to increase awareness Random lectures and information programs have to be introduced within the university campus. Administration should create art relating competitions with topics like climate change to increase awareness among universities. Seminars, lectures and information programs regarding global warming and environmental changes should be introduced time to time during the semester. Sessions should be organized by student affairs committee to create awareness among students in universities. Teachers and students should be asked about their opinions regarding the solutions one can perform to prevent climate change in universities. Prospective teachers should aware of global warming so that they can teach their students how to protect their environment. Not only prospective teachers Also peoples should be aware about global warming though Print and electronic media should play active role to provide awareness to public about global warming. People should aware be aware of global warming so that they can discipline their activities in order to protect environment.

Statement of conflict of interest
The authors have declared no conflict of interest.

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Sesquiterpene Lactones as Potential G2/M Phase Cell Cycle Checkpoints Inhibitors

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A B S T R A C T

Cancer is a second leading cause of death worldwide. Cancerous growth and abnormalities occur due to the dysregulation of cell cycle. Development of drugs to target cell cycle checkpoints leading to its arrest is a new paradigm for cancer treatment. Prevailing anti-cancer drugs are toxic, expensive and suffer from drug resistance. Therefore, idea of chemoprevention via natural products is getting more importance as these bioactive compounds are much safer, cost effective and provide potential alternative approach for treatment of cancer. In this study, interactions of G2/M checkpoints proteins (cyclin B1/CDK1) have been evaluated with seven bioactive compounds of sesquiterpene lactones (carabrolactone A, carabrolactone B, sulfocostunolide A and sulfocostunolide B, ascleposide E, ilicol and eucalyptone) family by using molecular docking tools to explore their cell cycle arresting potential. Outcomes of the present investigations reveal that carabrolactone B-Cyclin B1 complex and sulfocostunolide B-CDK1 complex exhibit the highest binding affinities of -8.2 and -8.0 kcal/mol with calculated inhibition constant values of 0.92 and 1.29 μM, respectively. All potential complexes have shown good molecular interactions including hydrogen bond. Drug likeness of the lead compounds was validated by ADMET analysis as well as Lipinski's rule of 5. Further *in vitro* and *in vivo* investigations are required to validate the inhibitory activity of studied sesquiterpene lactones to develop these compounds as novel potential G2/M phase inhibitors.

INTRODUCTION

Cancer stands second in causing most number of deaths worldwide. In 2023, about 1.9 million new cases and 609,360 fatalities are expected to occur in U.S. (Siegel *et al.*, 2023). A fundamental cause of cancer aberrations is the cell cycle dysregulation (Khan *et al.*, 2016). Cell cycle is a well-regulated event occurring under physiological conditions. This orderly process is under control of several regulatory proteins (cyclins and CDKs) called sensors or checkpoints. The stable complex of cyclin dependent kinase-1 (CDK1) and cyclin B1 (CB1) protein controls the transition of G2/M phase (Javed *et al.*, 2023; Gousias *et al.*, 2022). Under unfavorable conditions, this checkpoint is compromised and cancerous cell encounter uncontrolled proliferation. Targeted suppression of CDK1 and CB1 lead to the cell cycle arrest and thus cease the cancerous cells to proliferate, as reported by several studies (Sunada *et al.*, 2021). For that reason, profound understanding of regulatory mechanism of cell cycle can bring forth effective strategies for developing novel anti-cancer agents. Commercially available anti-cancer drugs are expensive and impose cytotoxic effect. Though plant based compounds are much safer, cost effective therefore, these compounds have been exploited for their anti-cancer potential (Khan *et al.*, 2015).

Sesquiterpene lactones (SLs) are wide-ranging class of plant based bioactive molecules, primarily extracted from Asteraceae plants that possess many biological activities (Paço *et al.*, 2022; Aliarab *et al.*, 2018). Their anti-cancer potential has been reported by several studies (Laurella *et al.*, 2022). Molecular docking (MD) provides an effective approach for the prediction and synthesis of novel compounds for evaluation of their pharmacological interactions and biological activities in conventional drug discovery (Asiamah *et al.*, 2023). The current study investigate interaction of seven ligands (carabrolactone A, carabrolactone B, sulfocostunolide A, sulfocostunolide B, ascleposide E, ilicol and eucalyptone) of SLs against G2/M checkpoints proteins as well as therapeutic potential of selected compounds against G2/M cell cycle arrest.

MATERIALS AND METHODS

Through MD, binding scores and ideal conformations of protein-ligand complex can be obtained (Fan *et al*., 2019; Morris and Lim-Wilby, 2008). For accurate docking analysis of protein-ligand interactions, AutoDock Vina (ADV) was used. In addition to that, PDBQT formats of both ligands and proteins were obtained by PyMol version 2.5.2 and MGL tools 1.5.7. Furthermore, the interactions of proteins and ligands were visualized and analyzed by BIOVIA Discovery Studio 2021 (Narkhede *et al*., 2020).

Retrieval and preparation of proteins

The 3-D structure of both checkpoint proteins, CB1 (PDB ID: 2B9R) and CDK1 (PDB ID: 4YC6), were retrieved from RCSB PDB database. Several modifications made to the protein structure includes removal of water molecules, addition of Kollman charges and polar hydrogen by using ADV. After these changes, protein structures were saved in PDBQT formats.

Retrieval and preparation of ligand

The 3-D structures of seven ligands were obtained in SDF formats from PubChem database. This format was then change to PDBQT format so that ADV can better compute it for further analysis.

Docking protocol

MD was performed by forming grid box by the dimensions of 40×40×40 and spacing of 0.375 Å. Lowest binding affinity of the complex determines the ideal conformation. Further interactions of the selected complex were further visualized and analyzed by BIOVIA.

ADMET analysis and drug likeness profiling

Absorption, distribution, metabolism, excretion and toxicity (ADMET) analysis was performed for the assessment of pharmacokinetic properties of ligands under study. For this purpose, pkCSM (online database server) was used for predicting ADMET properties and drug likeness profiling (Curreli *et al*., 2017; Pires *et al*., 2015). SMILES of proposed ligands were retrieved from PubChem, entered into pkCSM, and analyzed.

RESULTS AND DISCUSSION

Selection of ligand

SLs are abundantly found in medicinal as well as food plants. These compounds have many therapeutic potential including anti-cancer, antitumor, antioxidant and anti-inflammatory as reported by a plethora of studies (Karadeniz *et al*., 2021; Aliarab *et al*., 2018). Seven compounds of SLs

were selected and their chemical structures are shown in Figure 1.
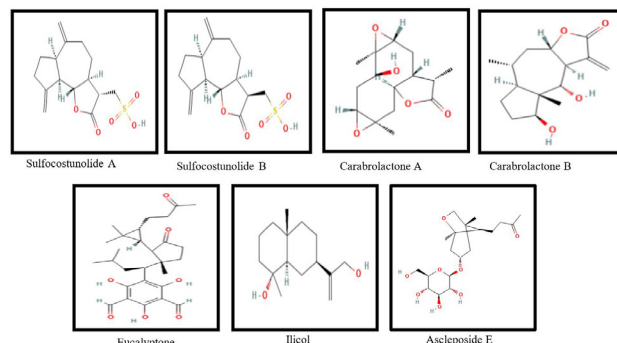
Fig. 1. Chemical structure of selected SLs.

Binding energy and inhibition constant

For exploration of novel drug candidates, understanding of protein and ligand binding mechanisms is prerequisite. For this purpose, MD has been extensively used for predicting binding scores and interactions occurring between protein and ligand (Du *et al*., 2016; Sousa *et al*., 2013). Docking of ligands was carried out with CB1 and CDK1. The crystal structures of both proteins are shown in Figure 2. According to docking results sulfocostunolide B exhibit highest binding score of -8.0 kcal/mol with inhibition constant (k_i) of 1.29 μM with CDK1 and carabrolactone B exhibit highest binding affinity of 8.2 kcal/mol with inhibition constant (k_i) of 0.92 μM with CB1 (Table I).
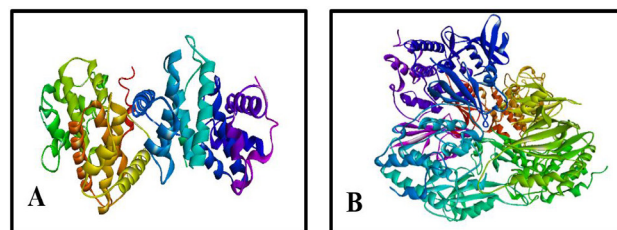
Fig. 2. Crystal structure of CB1 (A) and CDK1 (B) of G2/M phase checkpoints.

Protein and ligand interactions

Discovery studio visualizer was used for the visualization of interactions of protein-ligand complexes. According to our results, sulfocostunolide A and B binds successfully to CDK1. Whereas, carabrolactone B binds effectively with both proteins. Furthermore, these compounds showed good hydrogen and hydrophobic interactions with the proteins. Rest of the compounds showed less binding affinities with checkpoint proteins.

Table I. Binding scores, inhibition constants of CDK1 and CB1 with selected bioactive compounds.

Ligands	CB1		CDK1	
	Binding score (kcal/mol)	Inhibition constant (µM)	Binding score (kcal/mol)	Inhibition constant (µM)
Sulfocostunolide A	-6.6	13.8	-7.5	3.0
Sulfocostunolide B	-6.3	23.0	-8.0	1.29
Eucalyptone	-5.7	63.7	-7.0	7.0
Ilicol	-6.8	9.8	-6.4	19.4
Ascleposide E	-7.1	5.9	-7.1	5.9
Carabrolactone A	-6.6	13.8	-7.4	3.57
Carabrolactone B	-8.2	0.92	-7.6	2.5

CB1, cyclin B1; CDK1, cyclin Dependent Kinase-1.

Table II. Drug likeness prediction using online database server (pkCSM) for the selected ligands.

Ligands	Molecular weight < 500 (g/mol)	H-bond acceptor < 10	H-bond donors < 5	log P < 5
Sulfocostunolide A	312.387	4	1	1.9644
Sulfocostunolide B	312.387	4	1	1.9644
Eucalyptone	486.605	7	3	5.1849
Ilicol	238.371	2	2	2.8924
Ascleposide E	388.457	8	4	-0.2541
Carabrolactone A	282.336	5	1	1.0239
Carabrolactone B	266.337	4	2	1.2621

In case of CDK1, sulfocostunolide A forms hydrogen bonds with LYS88 (3.34Å), ARG20 (2.86Å), ARG20 (2.65Å), ARG20 (2.49Å) and GLN50 (3.36Å) as well as hydrophobic bonding with TYR8 having bond distance of 4.Å (Fig. 3). However, sulfocostunolide B while binding to the CDK1 forms hydrogen bonding including LYS30 (2.Å) and GLU12 (2.59Å) as well as hydrophobic bonding with ILE6 (5.20Å) and PRO25 (4.96Å) (Fig. 4).

Fig. 4. Sulfocostunolide B docked with binding pocket of CDK1 (A), Two-dimensional illustration of CDK1/ sulfocostunolide B (B).

Fig. 3. Sulfocostunolide A docked with binding pocket of CDK1 (A), Two-dimensional illustration of CDK1/ sulfocostunolide A (B).

Carabrolactone B potentially forms hydrogen bond with ARG135 (2.57Å) and MET172 (2.70Å) and hydrophobic interactions with VAL173 (3.59Å) and ILE180 (5.11Å) amino acid residues of chain B of CB1 (Fig. 5).
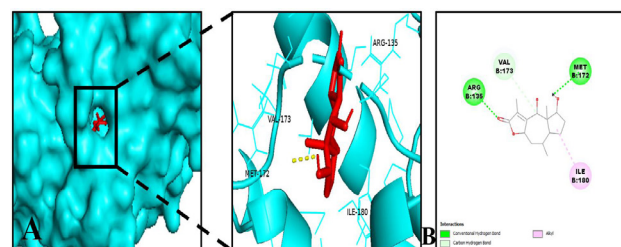
Fig. 5. Carabrolactone B docked with binding pocket of CB1 (A), Two-dimensional illustration of CB1/ carabrolactone B (B).

Table III. ADMET analysis of selected ligands using pkCSM online database server.

ADMET	Parameters	Sulfocos-tunolide A	Sulfocos-tunolide B	Euca-lyptone	Ilicol	Asclepo-side E	Carabrol-actone A	Carabrol-actone B
Absorbance	Water solubility (log S) mol/L	-2.185	-2.185	-3.977	-3.194	-2.938	-3.147	-2.709
	Intestinal absorption %	95.978	95.978	91.219	93.228	48.811	81.749	77.689
	P-glycoprotein I/II inhibitor	No	No	Yes	No	No	No	No
Distribution	log VDss (L/Kg)	-0.503	-0.503	0.068	0.306	-0.21	0.311	-0.147
Metabolism	CYP2D6 substrate	No	No	No	No	No	No	No
	CYP3A4 substrate	No	No	Yes	No	No	No	No
	CYP2D6 inhibitor	No	No	No	No	No	No	No
	CYP3A4 inhibitor	No	No	No	No	No	No	No
Excretion	Renal OCT2 substrate	No	No	No	No	No	No	No
Toxicity	AMES	No	No	No	No	No	Yes	Yes
	Hepatotoxicity	No	No	No	No	No	No	No

On the other hand, carabrolactone B forms hydrogen bond with GLN50 (2.63Å), GLN132 (2.17Å), GLN132 (1.92Å) and ASP86 (3.27Å) residues of CDK1 (Fig. 6). This docking data indicates formation of stable complexes of ligands and targeted proteins. Therefore, it can be concluded that the selected drug candidates might possess anti-cancer properties.
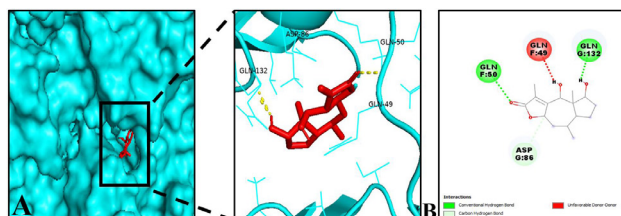
Fig. 6. Carabrolactone B docked with binding pocket of CDK1 (A), Two-dimensional illustration of CDK1/ carabrolactone B (B).

Drug likeness

For a drug candidate to be orally available it must have good absorption and fast excretion from the body. Therefore, drug likeness of drug candidates is predicted through Lipinski's rule of 5. This rule stated that, any ligand is considered to be drug-like if it is likely to have no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, molecular mass less than 500 Da and log P less than 5 (Lipinski, 2004). Except eucalyptone, all selected ligands obeyed this rule (Table II), which strongly suggests their suitability as a drug.

ADMET profile

Pharmacokinetic properties of drug compounds are predicted through ADMET (Absorption, distribution, metabolism, excretion and toxicity) analysis (Table III) which is essential for drug's bioavailability (Mathialagan *et al.*, 2017).

Absorption

Bioavailabilty of a drug is determined by its absorbance in small intestine (Radchenko *et al.*, 2016), which must be more than 30% (Flores-Holguín *et al.*, 2021). Absorbance of all the seven ligands exceed 30%. Additionally log S (water solubility) of all compounds is greater than -5 mol/L which is an indication of their solubility at 25°C in water (Pires *et al.*, 2015). If a drug is inhibiting P-glycoprotein I/II transporters it may cause interference with pharmacokinetics properties of other drugs (Lund *et al.*, 2017). However, our results reveals that only eucalyptone is P-glycoprotein I/II inhibitor while the rest of the drug molecules do not inhibit these proteins (Table III).

Distribution

Bioavailability of a drug is also determined by its uniform distribution (VDss) in blood plasma and its range value is -0.5 – 0.45. If VDss is high then it shows greater distribution in the plasma instead of tissue whereas low VDss value represents less diffusion across cell membranes (Llorach-Pares *et al.*, 2017). Current study shows that all ligands are ideal in terms of VDss (Table III).

Metabolism

Drug metabolizing enzymes can also affect the pharmacokinetic and pharmacodynamics properties of the drug. Any slight change in the activity of cytochrome P450 (detoxification enzyme) may hinder the pharmacokinetic

properties of the drug as the drugs are being metabolized by these enzymes. This detoxification enzyme has two isoforms: Cytochrome P3A4 and cytochrome P2D6 (Muhammad *et al*., 2021; Tracy *et al*., 2016). According to our results, only eucalyptone inhibits these enzymes. However, other ligands have no role in inhibition of the enzyme (Table III).

Excretion

Organic cation trasnsporter 2 (OCT2), a protein transporter, has vital role in the clearance of a drug. If a drug is a substrate of this protein, it may interact with its inhibitor (OCT2 inhibitor) which may lead to harmful reactions (Pires *et al*., 2015). It can be seen in Table III that no ligands are the substrate of OCT2.

Toxicity

AMES toxicity manifests the mutagenicity of a compound whereas hepatotoxicity predicts the potential of drug to cause liver injury (Muhammad *et al*., 2021). Table III reveals that none of the compound is hepatotoxic. However, carabrolactone A and B shows mutagenic potential which leads to its non-bioavailability. As it is an in silico analysis so its mutagenicity is indecisive. For that purpose, further experimental evidences are required for the confirmation of its mutagenicity.

CONCLUSION

A dynamic molecular docking study was carried out for G2/M phase checkpoints proteins with bioactive compounds to develop a novel anti-cancer therapeutic. Docking of potential ligands with CB1 and CDK1 revealed that the most stable complex of CDK1/sulfocostunolide B exhibit the highest binding affinity of -8.0 kcal/mol as well as good hydrogen and hydrophobic interactions. In addition to that, drug likeness profiling and ADMET analysis showed that the CDK1/sulfocostunolide B complex has efficacious pharmacokinetic properties. Based on the results, it can be suggested that this potential complex can lead to cell cycle arrest at G2/M phase. However, present study suggest further *in vitro* and *in vivo* investigations to develop sulfocostunolide B as a novel G2/M phase inhibitor.

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Mammal Survey of Thar District

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A B S T R A C T

This paper records and includes the list of trapped/netted small mammals and medium and large sized mammals observed nocturnally, with strong search-lights, during the 2015-16 survey and additional information gathered in second surveys of 2019 and 2020. A thorough review of the literature, related to each mammalian species recorded during the earlier as well as later surveys is given. Results of findings include the role of each species in the desert ecosystem of the study area is also discussed.

INTRODUCTION

This paper gives the results of two separate mammal surveys of district Thar, each conducted by the first author in 2015 in spring and again in autumn. Repeated again in 2016 in spring and autumn. The second similar surveys were conducted by both first and second author as a part of baseline study of the flora and fauna of district Thar, conducted in spring and autumn seasons in 2019 and 2020, by a consolidated team of the subject specialist, under a project of the International Union for the Conservation of Nature and Natural Resources (IUCN).

The first two years surveys were part of a bigger mammalian surveys of Thar Desert of Sindh that included the sandy desert on the eastern side of Nara Canal, South to the Indian border at the Rann of Kachh.

IUCN decided to make baseline survey of flora and its ecolinkages, mammals, birds, reptiles and amphibians, and land invertebrates, because Thar Coal Mines' commercial exploitation to produce electricity was first started and later on another company, perhaps not yet in power production. There are towers for high tension power transmission, roads and other infrastructure for coal mining and power production. There is lot of day and night heavy and light traffic on the new roads. The coal mining of Thar Desert has drawback of huge quantities of saline water on the bed of the coal. This water has to be pumped out constantly into a reservoir between the two sandy ranges with the construction of sand bunds on both edges. There are several trees growing in the inter-dunal flat land, which are now submerged in this saline lake. The mine owning company has introduced Talapia fish, which adapted so well that it is not only harvested for the company but also for the local poor communities. More than that, there is variety of fish-eating birds. These birds roost for the night on the living or dead trees. Their droppings keep on contributing in the fertility of the lake water. Since the desert air is dry, the fast evaporation brings cooling effect all around the lake. It was observed the species of vultures which have IUCN status of near extinction, were there under the trees at mid-day in very hot season.

All these obvious factors has brought a drastic change in the ecology of the southern Thar Desert District, which required to undertake Environment Impact Assessment. The second of 2019 and 2020 survey of this desert district was taken up by the IUCN Karachi, under the overall leadership of the second author and the technical leadership was given to the first author.

Ellerman and Morrison-Scott (1950) prepared a checklist of Palearctic and Indian Mammals, based on the

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collections in the British Museum of Natural History and related publications, which are also reviewed briefly. This checklist of 810 pages gives the diagnostic characters and distribution of each genus, species and subspecies.

(i) The species of order Insectivora that this checklist covers in the Thar Desert are *Hemiechinus auritus collaris.* This subspecies is now considered as Palearctic and not found in Thar desert, as has been given in this checklist. The species *Hemiechinus collaris blanfordi* is found in the Thar Desert.

(ii) *Paraechinus micropus* is mentioned as found in Thar Desert, whereas *Paraechinus micropus micropus* distribution is given from Bahawalpur and northern India.

(iii) Identification key for both genera and species is also given.

(iv) *Suncus murinus sindensis* covers Thar Desert, but Roberts (1997) does not show its distribution from Thar Desert.

(v) *Suncus stoliczkanus* distribution is included in the Sindh province, but its occurrence in the Thar District is not mentioned.

(vi) *Tadarida egyptiaca* is mentioned to cover Sindh province and eastward in India. The present study area covered in that.

(vii) *Rhinopoma microphyllum* is not reported from Pakistan in this checklist. It is recorded in subsequent studies from southern Sindh as well, which covers this study area.

(viii) This checklist has mentioned the following species as reported from Sindh including Thar Desert which includes present study area: *Scotopfhilus heathii; Millardia meltada pallidor; Millardia gleodowi; Rattus rattus.* Its global distribution is given, covering the Thar District *Rattus rattus rufiscens.*

(ix) *Mus booduga* its distribution is given in Punjab and Kutch. Sindh or Thar desert is not mentioned. However, later on it was reported from Thar Desert, covering present study area.

(x) *Cremnomys cutchicus* in this checklist it is reported as subgenus of *Rattus* and not reported from areas of Pakistan. However, later on it was discovered from Karoonjhar Hills at Nagarparkar, extreme south-eastern Sindh.

(xi) *Gerbillus gleodowi; Tatara indica indica; Meriones hurrianae; Canis lupus palipes* and *Canis aureus aureus* distribution from the study area in Sindh is mentioned.

(xii) *Vulpes vulpes* key to identification of subspecies and their distribution is given. *Vulpes vulpes* pusilla is the subspecies which is reported to cover the study area of Thar Desert.

(xiii) *Hyaena hyaena hyaena* subspecies is mentioned

to occur in Indian range of species is mentioned. *Felis sylvestris* is not reported from Pakistan. Instead *Felis lybicus* is mentioned to occur in Thar Desert. Subsequent publications mention the occurrence of the sub subspecies *Felis sylvestris ornate* in the Thar Desert.

(xiv) The distribution of *Felis caracal; Boselaphus tragocamelus* and *Gazella bennettii* from Thar is given in this checklist.

Siddiqui (1961) published a checklist of mammals of Pakistan with particular reference to the mammalian collection in the British Museum of Natural History (BMNH). This included all the species which were. Additionally, keys for identification and distribution of species were given. He erroneously included *Allactaga hotsoni* in the recorded list of mammals of West-Pakistan from the label of the study skin in the collection of that museum, which gave the locality 'Sib' Persian Balochistan, perhaps not knowing that this locality is in Iran. That was a Type female specimen, only one in the BMNH collection. Latter, senior author collected the second specimen from Noshki, Balochistan, which the first male specimen of the species, in the BMNH collections.

Mirza (1969) published the first book on mammals of west-Pakistan, which is the only book so far published in Urdu langue. It is mainly based on the secondary information of published literature. However, his own fieldwork of more than ten years enabled him to include four species to the recorded list of the mammals of West-Pakistan and added new information to the recorded distributions of the species (distribution maps in his book). He also added briefly, the ecozone of West Pakistan.

Roberts (1997) published first book in English Language, which gives comprehensive account of each species recorded from Pakistan (Former West-Pakistan). Prior to the species account he gives Pakistan's zoogeographical aspects and faunal origins, in the introduction of the book. This also includes brief account of the ecological zones of Pakistan, describing the flora, mainly the forest and the mammals found in them. This is 525 page (26 X 20 cm page size) double column book. The species account includes details of taxonomy, descriptions, illustrated by his own hand made black and white line-drawings, status, distributions with distribution maps. He has duly acknowledged the persons from whom he was benefitted in writing the text, including me. However, he erroneously wrote that I have mentioned in my book 'Small mammals of West-Pakistan' that the woolly flying squirrel *Eupaterious cinerius* is found in Ayubia National Park. When it was pointed out to him, he accepted his error in writing.

Khan (2012) stated Indian Jird *Merionnies hurrianae* as pest of agriculture. The senior author has observed the

Indian Desert Jird *Merionnies hurrianae* both in Cholistan and Thar deserts of Pakistan. Their habitats consist of mainly bushy areas with firm soil. He has never seen them living in or near agricultural areas, as against documented by the above-mentioned author, that their habitats are threatened due to the expansion of agriculture, and that, they also suffer from pest control programmes. On the contrary my observations are that this rodent is not a pest of agriculture. I have also not seen them to carry any food in their mouths for storge in the burrows. On one hand the above mentioned author has documented it as threatened due to its habitat loss because of the expansion of agricultural land, on the other hand it is declared as pest of agriculture.

This rodent is active during the day and sleeps at night. It feeds on seeds, roots, nuts, grasses and insects. Free water is not available to it, in the most part of the year. It must be getting its water requirements from moisture in the vegetation as well as its physiological ability to use the metabolic water produced during the oxidation of the food, for its physiological requirements, including the urination.

Roberts (2005) published a pocket size-size field guide to the small mammals of Pakistan. Again, in the same year another field guide to large mammals of Pakistan. This covered description of species, known distribution and habits. Each species is illustrated with photographs or line drawings. There is a distribution map of each species. In the field guide to the large mammals there is one perhaps printing error, that family Manidae of Order Pholidota is placed in Order Primates. Marine mammals have also been covered. Khan (2012) conducted a ten-day trip to study mammalian diversity in desert habitat of Tharparkar District, Sindh. Their observations were on nocturnal mammals, at night, with search-lights, in measured sample sizes. The study was conducted from April 1, 2013 to April 10, 2013. Microchiroptera were not included in the study. The number of mammals observed is not given, but their relative abundance is stated in the text. IUCN status of a list of 35 mammal species, is tabled. References of studies in the Thar Desert are given, without any review, as the location of study area is in the extreme south of this desert and merges with it northwards, where the habitats are significantly diverse. There is no comment on these habitats. However, examples of Rajasthan are given based on Indian literature. Literature based information on tropical thorn forest is given, but the issues of its degradation have not been touched. Population of Hyaena in Karoonjhar Hills appears to be exaggerated, as the daily number of bones around Nagarparkar, which is majority Hindu population, who do not eat meat, does not appear logically sufficient for such big population of Hyaena. The authors have not seen any meat shop where cattle meat can

be available. The bones of cattle are usually not consumed by humans as compared to goat bones. Karoonjhar Hills have no ungulates nor any predator, which may make the bones available for hyaenas. However, at the foot of this mountain there are locations, where Nilgai and Chinkara Gazelle may be found in very small numbers.

The background information about the Thar Desert is well documented, however, there is tendency to repeat, instead of giving the reference of the publications.

MATERIALS AND METHODS

The field investigations were done in February and repeated in October, in 2015 and again in 2016. It was a study in some detail, by using two mist-nets, each 35 ft long, for the bats collection. 40 folding Sherman traps were used for small mammals' collection. Peanut-butter was used as bait. Medium and large mammals were studied during the nights, using strong search-lights. The trapped small mammals had to be identified and released on the spot where collected. Few mammals study skins and skulls were prepared for authentic identification. After release of each one or more live-trapped animals of each species, sometime had to be spent to study behaviour of the released animals.

The decision for not repeating the same hectic exercise of netting and trapping again in the second survey.

Since the general conditions of the habitats remained the same, as were in 2015 and 2016, it was decided not to repeat the same exercise for this Thar District baseline study.

For trapping the small mammals, the habitats were randomly selected on both sides of Mithi-Nagarparkar road, suburbs of Nagarparkar, Karoonjhar Hill and rocky terrain north-east of Nagarparkar. In the sandy desert traps were laid in a line from top of high dunes range to the end of interdunal flatland. This included close to the local desert communities' huts and their arid rainfed agricultural fields. Trap laying was completed before sunset, in the evening. The larger mammals' surveys were conducted for two to three hours at night, just after the traps were laid. Two strong hand search lights were used, one on the right side and the second on the left side of 4X4 Land Cruiser. Two front beams of the vehicle, of course, helped in scanning the area ahead. Medium and large mammals were easy to identify as these were dazzled by strong beams of the search-lights.

Two mist-nets were used to catch small bats, wherever their abundance and low flights were noted.

On way back from medium and large mammal search, same wheel track was used. Trapped small mammals were carefully transferred to transparent plastic bags

for identification, in the field by the author before their release. After release the author watched their movement with the help of strong hand beam. Some specimens were taken back to the hotel room for making study skins to ensure identification. Hedgehogs were picked from ground for identification and then released.

RESULTS

Long-eared desert Hedgehog (Hemiechinus collaris)

Two were found in bushy area of the desert about 20 km short of Nagarparkar in February, 2015. In October 2015 six solitary hedgehogs were collected and were taken to the lodge where the field team was staying. These were kept in big plywood carton. They were given beetles to feed on. One day they were given a saw-scaled viper to eat. These just started eating the body of the living snake. While eating the entire bodies of the hedgehogs except their heads were hidden within the prickly skin.

In February 2016 no hedgehog was found. Perhaps these were still hibernating. In October three were sighted, but were not picked.

Role in the ecosystems: Hedgehogs helps to keep balance of animal species in the ecosystems through food chain.

Anderson's shrew (Suncus stoliczkanus)

In October 2016 one individual was trapped in suburb area of Mithi town. After identification it was released. Shrews have faster metabolic rate. The author had kept five *Suncus murinus* in a box for some days, on meat. They were not given any food for a couple of days. They ate each other and the last one also died because of hunger. Their vision is very weak but their olfactory sensation is acute. They sniff their prey. Mother shrew leads the young shrews for hunting in a train formation by holding each other's tails in their mouths. As the mother shrew approaches a prey, all young shrews attack the prey with their mother and start eating instantly.

Role in the ecosystems: These helps to keep balance of species in the ecosystems through food chain.

Large mouse-tail bat (Rhinopoma microphyllum)

It was netted one in February and two in October 2015, at the edge of a wetland with tree growth. In October 2016 three were netted at the same wetland.

Role in the ecosystems: It is estimated and documented that the Microchiroptera bats consume flying insects, almost equal to their own weight each night.

Egyptian free-tailed bat (Tadarida egyptiaca)

In October, 2015 two were netted and again in October 2016 six were netted near Mithi town suburbs.

Role in the ecosystems: Same as of all small bats.

Greater yellow bat (Scotopfhilus heathii)

In October, 2015 several of this species were found hidden in a wooden switch board of a house.

Role in the ecosystems: Same as all small bats.

Soft-furred field rat (Millardia meltada)

In October, 2015 one was trapped from an agricultural field close to a small village few km from Mithi on one side of the road to Nagarparkar.

Role in the ecosystems: It is a pest of 'Barani-agriculture'. Its role in in food chain is important as it breeds in spring as well as in post monsoon season. Each litter has 5-8 young ones.

Sand-coloured rat (Millardia gleodowi)

In each trip to sandy scrub areas on both sides of Mithi-Nagarparkar road during spring and post monsoon seasons in 2015 and 2016, three to six of these rats were always trapped and released after identification.

Role in the ecosystems: It is preyed upon by snakes, which enter its burrows, and carnivore mammals like foxes and Indian Desert Wild Cat. It breeds in spring and autumn season with smaller litter size of 2–3 young ones.

House rat (Rattus rattus)

It is so common, well-fed and happy in the hiding places in urban areas that it is well-known to public for its damage to food items, sometimes leather items, even it gnaws at wood items, it has ectoparasites that carry diseases, including the germs of 'Salmonella' in its urine. It was therefore decided not to waste time in its trapping. However, it was often cage-trapped, which had peanut butter as bait, in suburban areas.

Role in the ecosystems: It very successful human dependent species, with almost no enemies except that it is killed by humans to get rid of it. Domestic cats are usually well fed that they do not bother to kill these to eat.

Field mouse (Mus booduga)

It was mostly trapped in bushy habitats, particularly from thorny bushes close to agricultural areas. It feeds mainly on grains and seeds. It may eat insects if found nearby. It is swift and agile. It climbs the branches of bushes to probe for food. It is nocturnal and is vulnerable to nocturnal predators.

Role in the ecosystems: It breeds throughout the year and has litter size 4-8 or more. Hence, it is important in food chain.

Cutch rock rat (Cremnomys cutchicus)

It is confined to Karoonjhar Hill. Only one specimen was trapped near the temple in October, 2016. It is vegetarian. It may be attracted to eatables from garbage, as it came in the trap placed adjacent to a garbage pile. Certainly, it was attracted to peanut-butter's smell. It is nocturnal and is vulnerable to nocturnal predators

Role in the ecosystems: Its litter size is 2-8. It has normal value in the food chain.

Hairy-footed gerbil (Gerbillus gleadowi)

It was trapped from firm sandy habitats with bushes. It is nocturnal and finds seeds and grains from soil and soft leaves and grass. It also eats insects if available, particularly in post monsoon period.

Role in the ecosystems: It has two litters, one in spring and the second in post monsoon period. Each litter has 2-5 young ones. Its value in food chain is normal like other rodents.

Indian gerbil (Tatara indica)

In the Thar district the senior author trapped these from firm soil, closer to local communities' habitations. Some of its burrows were 'U' shaped. Water was poured in one opening and a male and a female came out of it. However, there were burrows with many openings. These are nocturnal and have many nocturnal enemies. In February 2015 four were trapped and in October in the same year seven were collected and released. They are very easy to identify from their grooved upper incisors and pail side stripes along the full length of the tail, which is bushy black at the end.

Role in the ecosystems: It breeds the whole year and produces 5 young per litter and 17 young annually. In post monsoon period it eats considerable quantity of insects. It is valuable for food chain in the ecosystems.

Indian desert jird (Meriones hurrianae)

It is social rodent living in colonies in firm sandy part of land, where many of these live in burrows made at the roots of bushes. These live on succulent plants and bark of shrubs, tubers, green plants, seeds and insects. They are active in the mornings and evenings. They do not live- in agricultural areas, nor were they seen collecting food from agricultural crops in their mouths. Yet they have been declared agricultural pests, assuming that since they are rodents so these must be the agricultural pests. They did not enter the Sherman trap, despite the liberal use of peanut butter.

Role in the ecosystems: Their litter size is four and each female has three to four litters a year. With this rate of population increase and the insect consumption in post monsoon season they are very valuable in food chain of the desert ecosystems. The senior author has seen foxes pouncing upon these as these come out of burrows.

Indian wolf (Canis lupus)

It was not sighted during the surveys. Local desert human communities reported occasional sightings of pairs and attacks on the goats. It is mainly nocturnal; It is also active in the mornings and dusk time. The pair chases its prey for long distances by running fast. They may catch a desert hare in a few minutes. The stray dogs are its easy prey. These dig deep dens or may enter porcupine burrows for day time rest. The local desert communities informed that there is no den nor there are porcupine burrows in the Thar district.

Role in the ecosystems: The female breeds once a year. 1-5 pups are born with closed eyes. It takes 10 to 15 days to get their eyes opened. They are wandering animals and may travel very long distances each day in search of food. They may not remain in one area for several days until the next breeding season. Their impact on food chain is variable in the ecosystems of an area. Generally, their role remains the same as that of other large predators, i.e., to contribute in creating balance of species in the ecosystems.

Golden jackal (Canis aureus)

During the surveys jackals were found in the vicinity of towns and sometimes also near the huts of the desert communities. They are nocturnal. They could not be accurately assessed as their sighting required driving with search light all around the towns, which was not possible. Their counting was dropped.

Role in the ecosystems: Jackals are mainly dependent on human communities, as scavengers of municipal wastes, including human excreta. They are also predators of small mammals and the eggs or chicks of ground nesting birds. They can be considered to play useful role in the ecosystems in the vicinity of urban habitats. Female gives birth to three to five pups. The Pups suffer higher mortality, often due to hunger and malnutrition. These also carry ectoparasites as well as endo-parasites. Perhaps diseases from these parasites also cause their mortality and help control of their populations.

Desert fox (Vulpes vulpes)

In February 2015 nine foxes were seen during the nights on both sides of Mithi- Nagarparkar road, during the trips in the desert, with search lights. They were all solitary. Closer to Nagarparkar, only four foxes were seen. (Nocturnal searchlight surveys were not allowed all around the town, due to security reasons, being closer to the Indian border).

In October 2015, 13 individuals were spotted on Mithi-Nagarparkar road, on the same site of the month of February.

In February 2016 in the same areas, only seven were sighted on Mithi- Nagarparkar road side trapping areas and three were seen near Nagarparkar.

In October 2016, 10 individuals were spotted on Mithi-Nagarparkar road, on the same February sites.

This fox is expert predator of small mammals. It is nocturnal. In 2015 during the long dry spell, rodent populations had reduced due to dried up bushes, particularly the herbs and grasses, the foxes appeared weak and lethargic. They were seen hunting Indian Desert Jirds in the morning time.

Role in the ecosystems: Foxes help in keeping balance of species in the ecosystems by controlling the prey populations.

Striped hyaena (Hyaena hyaena)

This nocturnal scavenger of the bones of the fresh dead animals is reported from Karoonjhar Hills. This area is sensitive, as it is adjacent to Indian border. Night Survey, with search lights was not allowed. It is adapted to get nutrition from the bones of freshly killed animals. Since there are no ungulates and no large predators to kill them, so, it has no food available to it in the wild. It is logical to assume that a very small population of this species must be living on the fresh bones of goats available in the municipal waste from Nagarparkar.

Role in the ecosystems: It is a scavenger.

Indian desert wild cat (Felis sylvestris)

In February 2015, while surveying with search lights more than 50 km north of Mithi-Nagarparkar road, by Chance it was spotted sitting on top branches of a Prosopis tree. It was dazzled with search lights and did not move. One of the field persons quietly climbed the tree with a hope to catch it. As it touched the hind leg of the cat, she hit the field man's head with her front paw, jumped down from the tree and disappeared in the scrubs. This was the only siting of the desert cat during the surveys.

Role in the ecosystems: This is a predator of small and medium size mammals. Its role is the same as those of other predators in the food chain of the desert ecosystems.

Caracal (Felis caracal)

It could not be sighted during the surveys. The local communities also did not confirm its occurrence un this desert. However, a Conservator of wildlife, who accompanied the survey team insisted that this cat still occurs in the Thar District.

Role in the ecosystems: Same as other medium size predators.

Nilgai (Boselaphus tragocamelus)

It is a nocturnal ungulate and reported to occur along Indian border fence. So, it could not be surveyed.

Role in the ecosystems: It is a large size prey species of large size predators. Humans have exterminated both large preys and large predators from the study area.

Chinkara (Gazella bennettii)

A landlord of large desert has a population of 70+ in small family parties of up to 5 or 6 animals in his private reserve, few kilometers away from Mithi. Elsewhere, It was not seen during the surveys in the entire district Thar. According to the district Wildlife Officers, it has been persecuted from this district except close to the border fence area.

Role in the ecosystems: It is a large prey species of large size predators.

DISCUSSION

Thar District (Mithi and Nagarparkar), like the entire Thar desert is highly populated area. Most people live in huts, wherever drinking water can be found. They are relatively neglected, particularly educationally. Like all backward areas of Pakistan, they do not practice family planning. The higher rate of growth of population and their traditional livestock rearing have negatively impacted the original floral set up of the typical natural habitats of the Thar Desert. Constant increase of livestock grazing has gradually eliminated or critically reduced the food plants of the domestic livestock as well as of the wild ungulates. That resulted in the reduction of all wild herbivores. As a result to that. And due to uncontrolled poaching, VIP and Royal hunting periods, all game species have been drastically reduced. All big and medium size carnivore mammals have also declined. So much so, that they are now confined to isolated small pockets. These are the salient ecological factors. Additionally, there are other factors like continuous reduction and degradation of the original thorn forests' ecozone. The coal mining and electric power production, roads, all types of infera structures and day and night traffic have made a great change in the ecology of this desert district.

CONCLUSION

Degradation of this desert has crossed the boundaries of no return. It is proposed that there should be a feasibility study to make a work plan to bring back the deteriorated state of environment to normal as much as possible.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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Artificial Nesting of Birds at University of Veterinary and Animal Sciences, Ravi Campus, Pattoki, Punjab, Pakistan

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A B S T R A C T

Spatial and anthropogenic factors influencing nest-site selection in birds need to be identified for effective conservative practices. Environmental attributes such as food availability, predator density, previous reproductive success, etc., provide site quality information. An artificial nest box experiment was carried out to evaluate the effect of various factors on nest-site selection, as it is hard to assess the natural cavities. Artificial nest boxes created with different materials, sizes, and colors were installed at different heights. The experiment was conducted whereby half of the boxes were filled with old nest material. A total of 14 out of 60 nest boxes were occupied, and four of them faced predation. The birds explored 32 out of 60 nests, whereas anthropogenic factors destroyed 25 out of 60 nests. Birds chose empty nest boxes at higher rates; however, there was no obvious avoidance of sites having high ectoparasites load due to old nest material. It is also possible that the preference towards the artificial nest boxes may differ yearly because of several climatic factors and the age of old nest material affecting the parasite survival. These variables may fluctuate from one season to another. Considering these factors, nest-site selection experiments concerning the effectiveness of artificial nest boxes should be carried out over several successive seasons. This topic may stimulate further studies, leading to a complete understanding of the birds' evolutionary ecology. Precise information on these factors influencing nest-site selection can be essential from an economic point of view as well.

INTRODUCTION

Most animals face diversified and, to a certain extent, unpredictable factors that affect fitness-related decisions during their lifetimes (Frey *et al*., 2016). One significant paternal decision for them is where to settle, which may impact an individual's survival. A female's decision to lay eggs at a particular place can severely affect her reproductive fitness and embryo survival (Refsnider and Janzen, 2010). Different bird species react differently to these factors during nest-site selection. Birds are known to be able to explore the environment for building nests, including the exploration of a diverse array of nest-building materials. Individuals may choose low-quality nests when no better option is available, making nest-site selection context-dependent (Stanback and Rockwell, 2003).

Birds can assess habitat quality using various informative cues (feces, feathers, old material, etc.). Such signals may be unavailable at the start of the nesting season, preventing birds from judging the nesting habitat quality. The type of information that birds can evaluate can be diverse, including conspecific behavior, predation risk, nest substrate quality, or a combination of those factors (Eggers *et al*., 2006). Some species that use social, public, and environmental information widely for colony-site settlement decisions and nest-site selection have been observed, such as raptors (Podofillini *et al*., 2018; Sumasgutner *et al*., 2014). Avian habitat selection and community structure are greatly affected by various factors, including food, interspecific competition, availability of nesting sites, and predation risk.

Nest predation is troubled by various habitat factors at multiple scales, and birds can enhance their nest success by speculating protected sites (Eggers *et al*., 2006;

Mönkkönen *et al*., 2007). For example, nesting activities near the habitat boundaries may increase the exposure of nests to the predator. Moreover, the design of artificial nest boxes is also influenced by predation risk, such as a larger entrance size attracting more predators (Le Roux *et al*., 2016). It is estimated that 2.9 billion birds in North America have been lost due to urbanization, fragmentation, climate change, and other landscape factors (Rosenberg *et al*., 2019).

The addition of artificial nest boxes has been successful in forests with a paucity of trees with hollows (Lindenmayer *et al*., 2009). The extensive use of artificial nest boxes has contributed to remarkable progress in our knowledge of physiology, behavior, and ecology. Cavity-nesting birds, especially small passerines, widely use artificial nest boxes. They provide an easy way for experimental observation, manipulation of eggs, risks of predation, identification of birds, and capturing birds for experiments and conservation (Lambrechts *et al*., 2017).

The old nest material in nest cavities (feces, prey remains, feathers, etc.) contains information about the previous breeding activity. It not only saves birds from material collecting efforts, but the material in the nest may increase thermal insulation and reduce egg heat loss (Mainwaring *et al*., 2014a; Mazgajski, 2007), potentially improving incubation efficiency. In contrast, empty nest boxes are considered low nest-site quality due to nest predation (Sumasgutner *et al*., 2014). Studies on ectoparasites load showed birds avoiding sites with old nest materials (Proctor and Owens, 2000). However, no consistent pattern has been observed that links the old nest material with nest-site selection in birds (Mazgajski, 2007).

Urbanization, the primary cause of biodiversity loss, influences the adaptability and survival of birds due to variations in the structure of vegetation cover, competition with exotic species, and predation risk (Jokimaki and Huhta, 2000). Biodiversity strategies compensate for the loss of habitual hollow-bearing trees removed during road and housing construction by adding the nest boxes on the smaller trees as replacement space for hollow-using fauna (Gibbons and Lindenmayer, 2007).

Understanding the factors that affect birds' communities using urban environments at both the patch and landscape levels is significant to effective management attempts to increase bird diversity (Xie *et al*., 2016). Previous studies have mainly covered the nest success and predation rate of artificial nest boxes installed on agricultural landscapes. However, comparing nest success and predation pressure between different landscapes may lead to an in-depth understanding of the impacts of man-made changes in landscape structures on avian community structures.

Ornithologists have not conducted extensive research on how various birds react to the old nest material, despite the relative ease of conducting such experiments. Therefore, this research aims to test the nest success and predation rate of artificial nest boxes installed at different sites and whether old nest material serves as an informative cue in nest-site selection in birds. We predicted that artificial nest boxes installed at areas of greater disturbance would experience lower nest success and greater predation rate. We also anticipated that if the old nest material is an informative cue in the nest-site selection, birds should choose the boxes with old nesting material and avoid the empty ones.

Table I. A summary of study predictions and their ecological justifications.

Factor	Prediction	Ecological justification
Entrance size	Nest boxes with larger entrance sizes will be occupied more than the smaller ones.	Small hollows are abundant in nature and hence are lesser in demand than the large cavities (Le Roux *et al*., 2016).
Landscape context	Nests installed at sites with dense vegetation and less human disturbance will be occupied more.	Birds prefer more wooded areas for nesting to minimize predation risks and human disturbance (Xu *et al*., 2020).
Tree height	Birds will prefer nest boxes secured at taller trees.	Nest boxes installed at taller trees experience lower predation risks and survive longer (Mainwaring *et al*., 2014a).
Nest dimensions	Smaller and covered nest boxes will be occupied more than the larger and exposed ones.	Birds occupy nests proportional to their body size, however, nest predation rates increase with the nest size (Mainwaring *et al.,* 2014).
Temperature	Birds will choose the nesting sites having optimal temperature.	Birds tend to select nesting sites that conserve or emit heat more efficiently and provide the optimal temperature for the offspring's development (Hansell, 2005).
Nest material	Birds will occupy nests constructed with wood.	Birds choose stiff and rigid material based on its structural properties; for nesting (Bailey *et al*., 2014).
Old nest material	Birds will occupy nests containing an old nest material.	Birds prefer nests with old nest material as it increases the incubation efficiency and reduces heat loss (Mainwaring *et al.,* 2014).

MATERIALS AND METHODS

Study site

The study was carried out from December 2020 to June 2021 at the University of Veterinary and Animal Sciences, C Block, Ravi Campus, Pattoki, Punjab, Pakistan (Latitude 31⁰ North, longitude 73⁰ East). The study site covered 263 acres with flat topography and an average elevation of 632 feet. The climate of the study area is temperate, characterized by sweltering, clear, and humid summers and short, calm, and dry winters. The temperature typically varies from 7°C to 40°C throughout the year, and the mean humidity level of the area during the study was 27.96%, and the recorded mean rainfall was 24.1mm (Pattoki Weather Forecast n.d.). The vegetation within the study area was diverse, and the height of the trees ranged from 15 to 56 feet. Dominant tree species of the study area include *Pongamia pinata, Melia azedarach,* and *Conocarpus*. The study site in the City of Flowers (Pattoki) hosts a wide variety of bird species due to its riverine habitat, heavy plantation of nearby Changa Manga Forest, and the Balloki Headworks. One hundred and two bird species were found in the C block, including residents, passage and summer migrants, and winter visitors (Ali *et al*., 2015).

Experimental design

We stratified our study area into five dominant landscapes representing varying degrees of anthropogenic activities, vegetation, and water bodies (Table II). Before deploying artificial nests, several surveys were conducted to gather data on different parameters such as longitude, latitude, area, and elevation using a GPS device. We randomly selected twelve trees in each landscape to install artificial nest boxes. The tree species were identified based on leaf color, shape, appearance, and texture. The trees' height, nests' height, and the DBH (diameter at breast height) were measured by climbing on the treetop and measuring the stem's length using a measuring tape following (Sumida *et al*., 2013).

A total of sixty artificial nest boxes of ten different types were constructed with wood, plastic, and clay. Wires, nails, plastic tape, and hot glue were used to assemble the body of the nest boxes, and the roof was carefully fixed to prevent the top from being opened unintentionally. The roof of each nest box was waterproof, and the bottom was designed to allow drainage. Each type of nest box comprised different widths, lengths, depths, and entrance sizes. Some of the nest boxes contained perch, a temporary resting place for birds. Each nest type was painted with a different color to determine the effect of color on the bird's nest-site selection (Table III).

Thirty pairs of artificial nests, i.e., a total of 60 in number (n= 60), depending upon their shapes, color, height, and structure, were introduced to the five selected landscapes with the help of iron wires, nails, and ladder (Fig. 1). The nest boxes were placed approximately 10-15 feet above the ground. Twelve nests were installed at each location, each pair of a different type. Nest-entrance orientation was determined for all nests by recording the line's direction from the centre of the nest box entrance with an ordinary compass (Mingju *et al*., 2019).

Table II. Description of the five landscapes.

Nesting sites	Description
Human settlements 1	Human settlement 1 is the area of the Department of Biological Sciences. Compared to the human settlement 2, this site features more greenery and lower levels of human activity. A poultry shed is located near it. Average nest height, tree height, and DBH were 18ft, 38ft, and 19.07cm, respectively.
Human settlements 2	Human settlement 2 is the area of the Department of Wildlife and Ecology. This site has the great hustle and bustle, including students, teachers, and workers. This site has a library, IT lab, cafeteria, fish hatchery, girls' hostel, faculty houses, and an academic block. This site has much human activity all day long due to playgrounds. Average nest height, tree height, and DBH were 15ft, 32ft, and 32.69cm, respectively.
Water bodies	The site where water ponds are present, which Fisheries Department supervises for fish rearing. These ponds are home to a large quantity of fish. The principal plants surrounding these water ponds are *Psidium guajava, Punica granatum, Mangifera indica,* and *Grewia asiatica*. Average nest height, tree height, and DBH were 14ft, 27ft, and 24.67cm, respectively.
Botanical garden	The botanical garden is located behind the fish-seed rearing unit. This garden was constructed in 2019 and had a variety of commercially important trees. This garden is surrounded by crop fields and is relatively unaffected by human activity. Average nest height, tree height, and DBH were 14ft, 22ft, and 44.29cm, respectively.
Road-side	This site is located near the main road and has rows of trees. A large number of traffic vehicles use this route, causing much disturbance. Average nest height, tree height, and DBH were 15ft, 29ft, and 21.23cm, respectively.

Table III. Description of ten different types of nest boxes installed at various landscapes.

Nest box type	Nest box code	Description
Rectangular box-wood	RBW	The rectangular wooden box had an entrance size of 5.7cm. It offered adequate space to accommodate larger birds. Length, width, and RBW depth were 7, 5.5, and 5.5 inches, respectively.
Large hut	LH	A large hut was made of wood and had an entrance size of 6.5 cm. It was painted with fresh aquamarine color to grab the attention of birds. Length, width, and LH's depth were 8, 7, and 7 inches, respectively.
Small hut	SH	A small wooden hut, with an entrance size of 5 cm, was painted with sea-green to attract birds. Length, width, and SH depth were 5, 5, and 5 inches, respectively.
Large pot	LP	A large pot was made of clay and had a rusty brown color. It had an entrance size of 6cm but lacked perch. LP's length, width, and depth were 5, 6, and 5 inches, respectively.
Medium pot	MP	A medium pot was made of clay and had an entrance size of 4.5 cm. Iron wires were used to assemble the cap with the pot. MP's length, width, and depth were 5, 6, and 6 inches, respectively.
Small pot	SP	A small pot was made of clay and had an entrance size of 4cm. Passerine birds prefer this size of nest box. Length, width, and SP's depth were 5, 5.5, and 4.5 inches, respectively.
Clay moneybox	CM	A money box made of clay was modified into a nest box. It had a dull brown color like wheat and an entrance size of 5.5 cm. Length, width, and CM's depth were 6, 8, and 6 inches, respectively.
Plastic pipe with cap	PPC	A wide plastic pipe was used to construct a nest, with its top covered by a waterproof cap. An entrance hole of 5 cm was present. PPC's length, width, and depth were 7, 4, and 6 inches, respectively.
Orange plastic box	OPl	A plastic box, orange in color, was modified into an artificial nest. It had an entrance size of 6cm and could host larger birds. OPl's length, width, and depth were 7, 5, and 5 inches, respectively.
Plastic pipe	PP	Plastic pipe, used to construct a nest box, was painted with a sapphire color. Its entrance size was 4.5 cm. PP's length, width, and depth were 6.5, 3, and 6 inches, respectively.
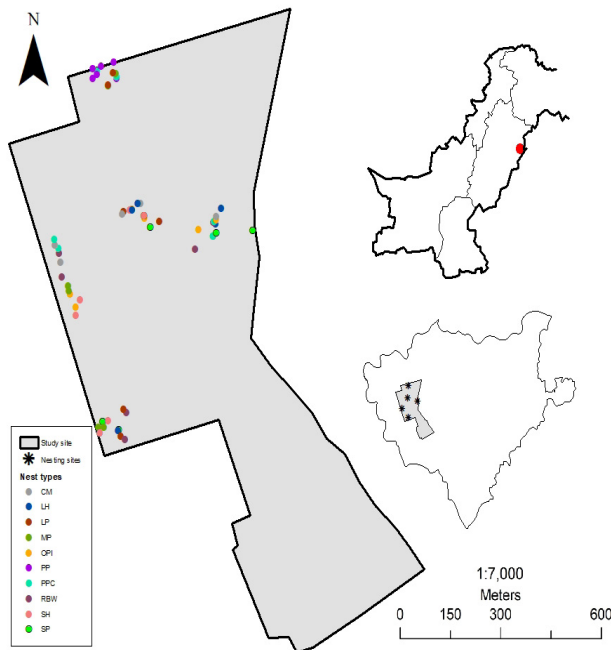
Fig. 1. Location and map of the study area in Pattoki, Punjab, Pakistan. The map depicts ten different types of nests installed at five sites, including the human settlements 1 and 2, water bodies, the botanical garden, and the road-side area.

One nest box from each pair was filled with a layer of old nest material previously used by bird species to determine the effect of old nest material on nest-site selection. Old nests of different bird species were collected from other areas far away from the study site. Collected nest material was kept in plastic bags at room temperature with a small opening for air exchange (Díaz-Lora et al., 2019). The old nest material made the boxes shallower, changing their cavity depth (Taylor and Mazgajski, 2010). Tree species carrying artificial nests were marked using tags, with nest numbers and signs indicating the presence or absence of old nest material accordingly.

Data collection

Nest boxes were inspected at intervals of 6-8 days to determine occupancy, signs of predation, or any other changes if present. Therefore, we conducted a total of twenty-seven surveys per landscape. Nest boxes were observed using binoculars to avoid interference with the nests. The term "occupancy" was defined as a bird present within the nest box at the time of inspection or conclusive evidence of current occupancies such as nesting material or eggs (Le Roux et al., 2016). We removed all depredated nests upon discovering predation evidence, such as the presence of feathers of the predator or the removal of eggs etc. Main predator groups were identified based on

the markings left and additional evidence found in the surrounding area.

Statistical analysis

We combined the data from the six months to calculate birds occupation and predation rate. We counted the number of artificial nest boxes containing different types of old nest material separately. The empty nest boxes were also counted to determine birds preference towards the presence or absence of old nest material (Table IV).

Table IV. Summary of categorical variables used in the study.

Variable	Categories	n	Nest success	Nest predation
Landscape[a]	HS1	12	2(3.3%)	0(0.0%)
	HS2	12	3(5.0%)	1(1.7%)
	WB	12	5(8.3%)	3(5.0%)
	BG	12	2(3.3%)	0(0.0%)
	RS	12	2(3.3%)	1(1.7%)
Nest material	Clay	23	6(10.0%)	3(5.0%)
	Plastic	19	2(3.3%)	1(1.7%)
	Wood	18	6(10.0%)	1(1.7%)
Old nest	Present	30	5(8.3%)	1(1.7%)
	Absent	30	9(15.0%)	4(6.7%)
Type of old nest	Baya weaver	10	2(3.3%)	0(0.0%)
	House sparrow	10	2(3.3%)	1(1.7%)
	Bulbul	10	1(1.7%)	0(0.0%)
	Empty	30	9(15.0%)	4(6.7%)
Perch	Present	12	4(6.7%)	1(1.7%)
	Absent	48	10(16.7%)	4(6.7%)
Direction	East	8	1(1.7%)	1(1.7%)
	West	16	5(8.3%)	3(5.0%)
	North	19	5(8.3%)	1(1.7%)
	South	17	3(5.0%)	0(0.0%)
Color	Brown	6	1(1.7%)	1(1.7%)
	Sandy brown	6	3(5.0%)	0(0.0%)
	Aquamarine	6	2(3.3%)	0(0.0%)
	Sea green	6	1(1.7%)	1(1.7%)
	Rust	6	1(1.7%)	2(3.3%)
	Natural wood	6	3(5.0%)	0(0.0%)
	Orange	6	0(0.0%)	0(0.0%)
	Cobalt blue	6	0(0.0%)	0(0.0%)
	Wheat	6	2(3.3%)	0(0.0%)
	Sapphire	6	1(1.7%)	1(1.7%)

a HS1, Human settlements 1; HS2, Human settlements 2; WB, Water bodies; BG, Botanical garden; RS, Roadside.

Factors affecting overall occupancy

An information-theoretic approach was used to select the best fit model by ranking models based on Akaike's Information Criterion values (AIC). We used generalized linear mixed models (GLMM) with a logit link and binomial error distribution to determine which factor best discriminated between occupied and unoccupied nest boxes. The dependent variable was the nest success (1 for occupied and 0 for unoccupied). The independent variables in the best model included the temperature, nest box height from the ground, the entrance size of nest boxes, dimensions of nest boxes, tree height, DBH, the material of nest boxes (1, 2, 3 for clay, wood, and plastic respectively), the color of artificial nest boxes (Sandy-brown= 1, Aquamarine= 2, Natural wood= 3, Rust= 4, Brown=5, Sea green= 6, Wheat color= 7, Sapphire= 8, Cobalt blue= 9, Orange= 10), and the landscapes where the nest boxes were located (HS1=1, HS2=2, WB=3, BG=4, RS=5). The R^2 values of the GLMMs were calculated using the MuMIn package of Rstudio.

Factors affecting the predation rate

We used GLMM (lme4 library in R) to analyze different variables' effects on nest predation. Nest predation was interpreted as a binary dependent variable (1 for predated nest and 0 for non-predated), and independent variables were selected based on AIC values.

Effect of old nest material

A multinomial logistic regression (Multinom function in the nnet R package) was used as the statistical method to identify the effect of the type of old nest material on nest-site selection. Occupation by the birds (1 and 0 for occupied and unoccupied, respectively) was included as the dependent variable, and the type of old nest material (Empty=0, House sparrow=1, Baya weaver=2, Bulbul=3) was included as the independent variable. Likelihood ratio tests were used to obtain *p* values (Table V). All of the statistical analyses were performed in R 4.1.0.

Table V. Summary of continuous variables included in the study.

Variable	Range	Mean	Std. deviation
Temperature (°C)	10-40	26.85	8.82
Tree height (m)	15-56	29.72	9.08
Nest height (m)	10-23	15.10	3.15
Entrance size	4.0-6.5	5.27	0.76
DBH	8.1-50.2	25.26	8.71
Dimensions	15-22	17.25	2.14
Depth	4.5-7	5.60	0.70

RESULTS

During this study, 14 (23.2%; Table IV) nest boxes were occupied by house sparrow *Passer domesticus,*

Indian magpie robin *Copsychus saularis,* common myna *Acridotheres tristis*, etc. Only five out of 14 nest boxes were predated (Table IV). Out of five diverse landscapes, nests installed at water bodies' sites were most occupied. Artificial nest boxes of clay and wood occupied more (10%) than plastic ones (3.3%). Birds chose empty boxes and old nest material from house sparrows and baya weavers at higher rates (Fig. 4). Birds explored 32 out of 60 nests. Activities (position and direction change) were observed in 19 nest boxes. Anthropogenic factors such as collecting the nest for pleasure, cutting trees, using tractors, and climatic events (heavy rains and thunderstorms) destroyed 25 of 60 nests.

Table VI. GLMMs used to determine the factors that affected whether birds occupied or did not occupy the boxes (Final model R²= 0.9756933, R²c= 0.9756933, n= 60).

Factors	df	Estimate	SE	X²	p
Temperature	9	0.092	0.039	24.461	0.0205
Landscape					
WBᵃ	4	2.327	1.226	.744	0.0578
Nest entrance	6	1.797	2.437	5.331	0.4608
Tree height	28	0.083	0.083	23.2	0.3186
Dimensions	6	-0.397	0.473	3.170	0.4002
Old nest material	1	-0.950	1.143	0.648	0.4054
Nest color					
Natural wood	9	2.880	1.919	7.493	0.1335

a WB, Water bodies.

Factors affecting overall occupancy

The temperature (GLMM: X^2 = 24.461, p =0.0205; Table VI) and the landscape context (GLMM: X^2 = 2.7445, p = 0.0578; Table VI) discriminated nest boxes occupied by the birds from unoccupied nest boxes. In contrast, the entrance/ opening size of nest boxes, dimensions, tree height, DBH of trees, presence or absence of old nest material, and the colors of nest boxes were not significantly associated with nest occupation.

Factors affecting the predation rate

Our experiment showed that none of the variables significantly affected the predation rate (Table VII). Only five out of 60 nest boxes were predated by hawks *Buteos* and crow *Corvus* during the study period (Figs. 2 and 3).

Effect of old nest material

In this study, the occupation rates of boxes containing different types of old nest material significantly varied

(Multinomial logistic regression, X^2 = 79.630665). Birds chose empty nest boxes at higher rates: 15% (9 out of 60; Fig. 4).

Fig. 2. Fallen Robin egg on the ground at the waterbodies site.

Fig. 3. Chick fell on the ground on the botanical garden site.
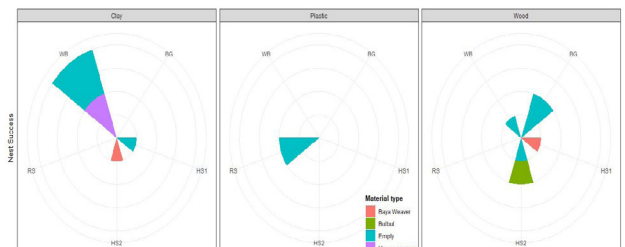
Fig. 4. The number of artificial nest boxes placed at different landscapes with different old nest types.

31m. A similar study for smaller, medium and large-sized trees suggested that birds find larger trees more attractive. Although large trees are essential for birds, they take many years to grow. Due to urbanization, old trees are cutting very rapidly. One of the vital advantages of old trees is that they provide natural hollows for the birds. Small and medium-sized trees do not attract cavity-nesting birds (Roux *et al*., 2016). The preservation of older trees requires proper management and regulation. The occupancy of artificial nest boxes could be increased by using nest boxes with different entrance sizes for different hollow nesting species. Targeting occupancy depends upon landscape context. It is not majorly affected by tree sizes (Le *et al*., 2016).
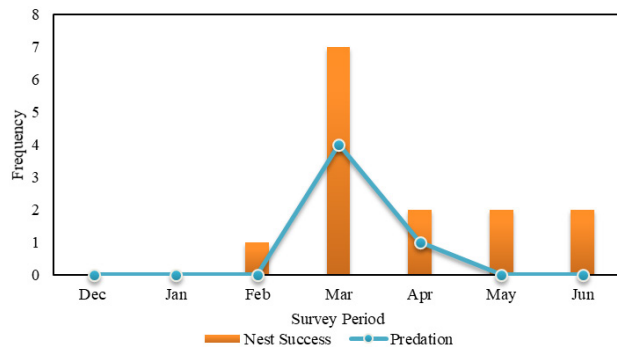
Fig. 6. Number of occupied nest boxes that faced predation during the survey period.

Another critical determinant of nest success is selecting a safe nesting site. Some birds have appeared to choose a location for nesting in places with a lower predation risk (Mainwaring *et al*., 2014a). Our study did not show any significant impact of included factors on the predation of nest boxes. This might be due to the fewer nest successes during the season. Nests installed at the waterbodies site were most predated, suggesting that the site is a suitable landscape for supporting various species. However, one out of two occupied nests at the road site also faced predation. A maximum number of predatory events occurred in March (Fig. 6). Several studies support that nesting activities near the habitat boundaries may increase the exposure of nests towards the predator. The exposed nests are more predated. Increased level of predation of artificial nest boxes was examined near the habitat boundaries in forest patches (Hartley and Hunter, 2008), in open grasslands habitats, and the nests in marshlands. Hence, avoidance behavior could minimize the influence of nest predation risk. Another study explores the effect of small woodland patches on the nest success and predation of northern lapwing *Vanellus vanellus.* Only

12% of Lapwing nests are within the 500m range and approximately 30% out of the 500m range. But still, 53% were predated by their predators (Bertholdt *et al*., 2017). This random nesting survey revealed that the predation rate does not alter with an increase in the distance from woodland patches. Rivera-López and MacGregor-Fors (2016) studied urban predation. They suggested that predation relaxation and intensification in the same urban system reveal that nest predation dynamics can be diverse throughout metropolitan areas. Moreover, Fokkema *et al*. (2018) studied the effect of nest-box depth on predation risk and breeding success of blue tits. Results showed no evidence of predation signs related to nest-box depth. However, apparent positive effects of nest box depth on the clutch size and hatching success were found.

Studies have provided a shred of conflicting evidence regarding the preference of birds towards old nest material (Podofillini *et al*., 2018); however, no consistent pattern has been found indicating the correlation between the presence or absence of old nest material and the nest-site selection (Mazgajski, 2007). An inclination of birds towards old nest material has been observed in some passerine species and raptors (Podofillini *et al*., 2018; Sumasgutner *et al*., 2014). Our study's results indicate birds preference towards empty nest boxes. One possible explanation for choosing empty nest boxes can be the depth of the nest boxes. Birds prefer deeper nest boxes regardless of the presence or absence of old nest material. Previous studies have found a relationship between nest depth, entrance size and nesting success (Wesolowski, 2002). Preference for empty nest boxes could also be a strategy to avoid ectoparasites in the old nest material. Old nest material contains more ectoparasites load, such as fleas or mites, than empty nest boxes (Mazgajski, 2007). Another possible reason for such preference may be birds' cleaning behaviour to reduce ectoparasite load and increase nest cavity. As it is time-taking and energy-consuming, birds prefer empty and clean nest boxes over ones with old nest material (Mazgajski, 2007). Thus, the preference towards empty nest boxes may be related to minimizing the workload for preparing a clean nest site. The presence of old nest material reflects the signs of successful breeding in the previous activity (Sumasgutner *et al*., 2014) and, as a result, increases the risks of predation. Birds tend to choose empty nest boxes to reduce the predation risk.

Studies reveal that sedentary birds like the great tits *Parus major* prefer empty nest boxes as they have sufficient time to assess nesting sites. Hence, such species do not require any cue to select the nesting sites, such as old nest material. Whereas migratory birds such as migratory pied flycatcher *Ficedula hypoleuca* arrives late at their breeding site and frequently use the nest sites

containing old nest material (Mazgajski, 2007). The results of our study evaluated that birds occupied the old nest material of baya weaver at a greater rate, which helped increase the thermoregulation and incubation efficiency of the nesting sites. Studies demonstrated that the old nest material provides favorable bird conditions, especially in extreme conditions (Hilton *et al*., 2004). Secondary cavity-nesting bird species lay their eggs on old nest material as it minimizes the energetic loss of incubation (Mainwaring *et al*., 2014). In burrowing owls, *Athene cunicularia*, removing the old nest material reduced nest box occupancy in subsequent breeding seasons, with birds returning from migration avoiding cleaned nest boxes (Riding and Belthoff, 2015). Similarly, female Eurasian kestrels *Falco tinnunculus* indicated a strong preference towards old nest material and laid eggs late in experimentally cleaned nest boxes (Sumasgutner *et al*., 2014). An inclination for nest boxes with old nest material in some passerine birds was observed, such as the pied flycatcher *Ficedula hypoleuca* (Olsson and Allander, 1995), the house wren *Troglodytes aedon* (Thompson and Neill, 1991), and the eastern bluebird *Sialia sialis*.

CONCLUSION

In conclusion, our research highlights the need for further investigation to enhance the effectiveness of artificial nest boxes. By exploring different sizes and designs and carefully selecting appropriate landscapes for nest box deployment, we can encourage a wider range of species to utilize these artificial structures. This approach holds tremendous potential for wildlife management and conservation efforts. Furthermore, our study suggests that future research should delve into the impact of old nest material on birds' evolutionary ecology. Gaining precise insights into how previous breeding seasons' nest material influences nest-site selection and current reproductive outcomes could prove invaluable both in terms of scientific inquiry and economic considerations. The financial resources currently expended on the annual cleaning of nest boxes in wooded and forested areas could be redirected towards other avian species or environmental protection activities if we better understand the effects of old nest material. It is important to acknowledge that the effectiveness of artificial nest boxes as a conservation tool remains a subject of debate. Therefore, empirical research is imperative to inform significant decision-making in this regard. By conducting further studies, we can better comprehend the true potential and limitations of artificial nest boxes, ultimately enabling more informed and effective conservation practices. Overall, our findings call for continued exploration and research, which can

contribute to the ongoing improvement and optimization of artificial nest boxes as an integral component of wildlife management and conservation strategies.

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The Impact of Elevated Arsenic Concentrations in Drinking Water on the Development of Type 2 Diabetes Mellitus

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A B S T R A C T

Drinking water of Kasur and Lahore is exposed to heavy metals (lead, chromium, and arsenic). Arsenic is most prominent in producing its carcinogenic and non-carcinogenic effects. Industrial wastes, agricultural activities and groundwater are sources of contamination of arsenic. The present study aims to investigate the role of elevated arsenic concentration in drinking water as a potential risk factor for developing type 2 diabetes mellitus. Sociodemographic data were collected through a questionnaire. For arsenic level testing, water and urine samples were analyzed with atomic adsorption spectroscopy. Blood glucose levels were evaluated through BSF, BSR and Hb1Ac. We found that Kasur has concentration ranges from 0μgm/L-116μgm/L while Lahore has a level of arsenic ranges from 0.88 μgm/L-70 μgm/L. The areas with a high concentration of arsenic had high blood glucose levels in the human blood. According to the present studies, there is an association between arsenic levels in drinking water and an increased risk of type 2 diabetes.

INTRODUCTION

Water is the simplest and fundamental component of the human body and its environment. It links living and non-living organisms to maintain balance on Earth (Chaplin and Chaplin, 2001; Li *et al*., 2019). Water plays a vital role in the sustainability of life; at the same time, it contributes a lot to the distribution of many diseases, which causes a prominent number of deaths globally (WHO, 2019). Many anthropogenic activities, such as deforestation for agricultural expansion and urban extension, are lowering drinking water quality (de-Mello *et al*., 2020).

Central Asia faces significant water pollution challenges. Agriculture, mining and irrigation are among the most common human activities that pollute surface water and groundwater. In the Asian Gold Belt, heavy metals and radionuclides are the most prevalent pollutants in water (Liu *et al*., 2021). Water pollution and scarcity mainly result from domestic sewage, industrial wastewater, storm runoff and agricultural practices. The industrial contribution of toxic chemicals (radioactive, organic, inorganic, acids, dyes, alkalies etc.) affects the human environment and health (Chowdhary *et al*., 2020).

Arsenic is an odourless and tasteless toxic metalloid which are found in four common valence states such as As(o), As(III), As(V) and arsine gas (Kuivenhoven and Mason, 2019). The metalloid arsenic is found in rocks, soils, water, and the air. Arsenic naturally occurs as arsenite or arsenate, depending on the oxidation and reduction conditions. It is used as a wood preservative, insecticides, pesticides, etc. It causes multiple diseases in the human body depending on the type and duration of their exposure (Pooja *et al*., 2020).

Arsenic is rare, only 0.00005% in Earth's crust. With an average of 6 mg/Kg of arsenic, different countries have different soil contents (ranging from 0.1-40 mg/kg). Pyrite has the highest concentration of arsenic, about 5 to 10% by weight. Some microbes also contribute arsenic in the environment by respiration.

About 108 countries, with 230 million people, which includes 180 million only from Asian countries, are at risk of arsenic exposure. Bangladesh was highly affected (Shaji *et al*., 2021). About 50-60 million people in Pakistan use arsenic water above 50 micrograms per litre (Podgorsk and Berg, 2020). Lahore and Multan, industrial waste is the most significant cause of arsenic in drinking water (Hussain *et al*., 2019). Naturally, arsenic concentration is

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high across the Indus River basin, which poses a permanent threat to human health (Qaiser *et al*., 2023).

Arsenic is among those metalloids that metabolize inside the human body and are absorbed through the digestive tract after food or water consumption (Flora, 2020). The breakdown of arsenic compounds, referred to as arsenic digestion, may undergo a series of chemical transformations due to an acidic environment (Yang *et al*., 2020). Most of the digestion takes place in the intestines (Coryell *et al*., 2019). Enzyme, i.e., arsenate reductase, converts the arsenate into more toxic arsenite (Mishra *et al*., 2022). The intermediate metabolites are more toxic, depending on their exposure source and trivalent biotransformation to prevalent (Luvonga *et al*., 2020). Gut microbiota plays an important role in arsenic metabolism using the reduction or methylation process (Wang *et al*., 2022). Arsenic methylation is an enzymatic reaction in which methyl group ($-CH_3$) is added to inorganic arsenic or its compounds. The liver is the site of arsenic metabolism, and its metabolites excrete through urine. The methylation process converts inorganic arsenic into organic arsenic metabolites considered less toxic and easily excreted from the body (Stýblo *et al*., 2021). Arsenic methylation is affected by various factors, including arsenic form, nutritional factors, exposure level, gut microbiota and genetic factors (El-Ghiaty and El-Kady, 2023).

Humans are exposed directly (inhaling and drinking water) and indirectly (through the food chain) to arsenic. Arsenic toxicity can cause hyperkeratosis, cancer and vascular diseases. The body excretes arsenic through breath, skin, urine, and hair. Urinary arsenic indicates arsenic poisoning (Othman *et al*., 2021).

Chronic exposure to elevated levels of arsenic in food and water causes numerous health problems. Strong evidence showed that arsenic exposure through groundwater is associated with developing type 2 diabetes mellitus. It has been suggested that arsenic exposure results in insulin resistance, improper glucose metabolism, and malfunctioning of the pancreatic beta cells. Arsenic with different oxidation states generates reactive oxygen species (ROS) and interferes with the normal functioning of natural oxidants. In this way, arsenic cause pancreatic beta-cell dysfunction, a major cause of insulin resistivity, leading to diabetes mellitus (Bao *et al*., 2019).

The current study aims to investigate the impact of elevated arsenic levels in drinking water on the development of diabetes mellitus type 2. The objectives include assessing the arsenic levels in the drinking water of selected areas, arsenic levels in the blood and urine of people exposed to arsenic and evaluating the association between arsenic exposure and diabetes mellitus type 2.

MATERIALS AND METHODS

Study areas subjects

This study was conducted in districts Lahore and Kasur of Punjab province. Sheikhupura District at the northwest boundary of Lahore, Wagah at the East and Kasur District at the south. The total area of Lahore is 1014 Km^2. Mostly the population of Lahore is Urban settlements because Lahore is 2nd largest metropolitan city in Pakistan, 5th largest city in South Asia and the 26th largest city in the world.

Inclusion criteria: participants (i) having type 2 diabetes, (ii) who were aged between 30-80 years and (iii) belonged to all ethnic groups.

Exclusion criteria: participants who were (i) less than 30 years and more than 80 years of age, (ii) suffering from major complications, (iii) pregnant women, (iv) with major surgeries, and (v) taking some multivitamins.

Data collection

A well-structured questionnaire was designed for the data collection from the participants. The questionnaire gives all the information which is required for the study. We request the participants to read the information attached to a questionnaire (for ease urdu version is also attached), which includes the objectives and aims of our study and has written permission for the data publication. After the willingness, a unique patient ID was given according to area name and district (Kasur and Lahore). The questionnaire was completed with the help of research assistants, facilitating the illiterate and aged participants.

Samples collection

Water, blood and urine samples from the same person were collected with the help of an expert technician. Every sample was given the same ID allotted to the participant.

Water sample

The water tap was opened at its full flow for 10-15 min, depending on the usage of tap. Then open water container was filled with water avoiding any touching container and labelled the container with ID, date and time. The water samples were stored in ice box and later to freezer for testing.

Blood sample

With the help of an expert technician, blood samples were collected after 12h of fasting by Venipuncture. Blood was shifted to serum separator tubes. Serum was allowed to centrifuge at 2,500 rpm for 15 min and later stored at -80ºC.

Urine sample

Sterile, polythene, screw-cap was used for first

catch urine samples collection and properly labelled. The container was placed in a plastic container and then in ice box for future testing.

Estimation of arsenic level

Stored water samples were assessed at the University of Punjab for arsenic detection. All the chemical required for analysis was available in the laboratory. An atomic absorption spectrophotometer measured arsenic concentration. AAS is a very common method for the detection of heavy metals.

To detect arsenic in water samples, a 100 ml sample was mixed with 2 mL of 30% H_2O_2 and a few drops of concentrated HNO_3. The solution was heated to 95°C until digestion, reducing the volume to 50 mL. After cooling, the solution was transferred to a volumetric flask and the reagent was added. To create a 1% nickel nitrate solution, 5 mL of the digested solution was pipetted into a 10 mL flask and filled with reagent water. The resulting solution was then used for further arsenic detection (Arshad *et al*., 2016).

The blood sample of 0.5 mL and concentrated HNO_3 (1 mL) were added to the digestion flask. The mixture was heated to 100°C and fermented for 40 minutes. Further, 0.35 mL of concentrated $HClO_4$ and H_2SO_4 were added to the flask. For optimal absorption, the solution was stored for 60 minutes. To dilute the solution in the digestion flask, 0.5 mL of deionized water was added. A colourless solution was then achieved by heating the flask. Additional testing and research were conducted using this diluted solution (Neha *et al*., 2013).

The colourimetric Merck kit, ranging from 0.02 to 3 mg/L, was used to check arsenic concentration in drinking water and urine.

Estimation of glucose level

To measure the blood glucose level, we utilized standard procedures to determine fasting blood sugar, random blood sugar, and Hb1Ac. To measure blood sugar levels, a test strip with blood on it is inserted into a Glucometer. It is type of dry chemistry (the specimen is applied to strips that have been impregnated with dry reagents).

Statistical analysis

Data wa tabulated into excel sheets. Later data was imported to SPSS for analysis. t test was applied on data and p value was calculated for significant association

RESULTS

Analysis of subjects involved in the study

In Kasur, 1% were below 30 years, and 18.4% had between 30-40 years, 20.8% had age between 40-50 years, and 59.8% were above 50 years. In Lahore, only 0.4% had an age below 30 years, 20.8% were between 30-40 years, 20% were between 40-50 years, and 58.4% had age above 50 years (Fig. 1A). From Kasur, 40% were males and 60.5 were females. Similarly, from Lahore, 38.6% were males, and 61.4% were females (Fig. 1B).
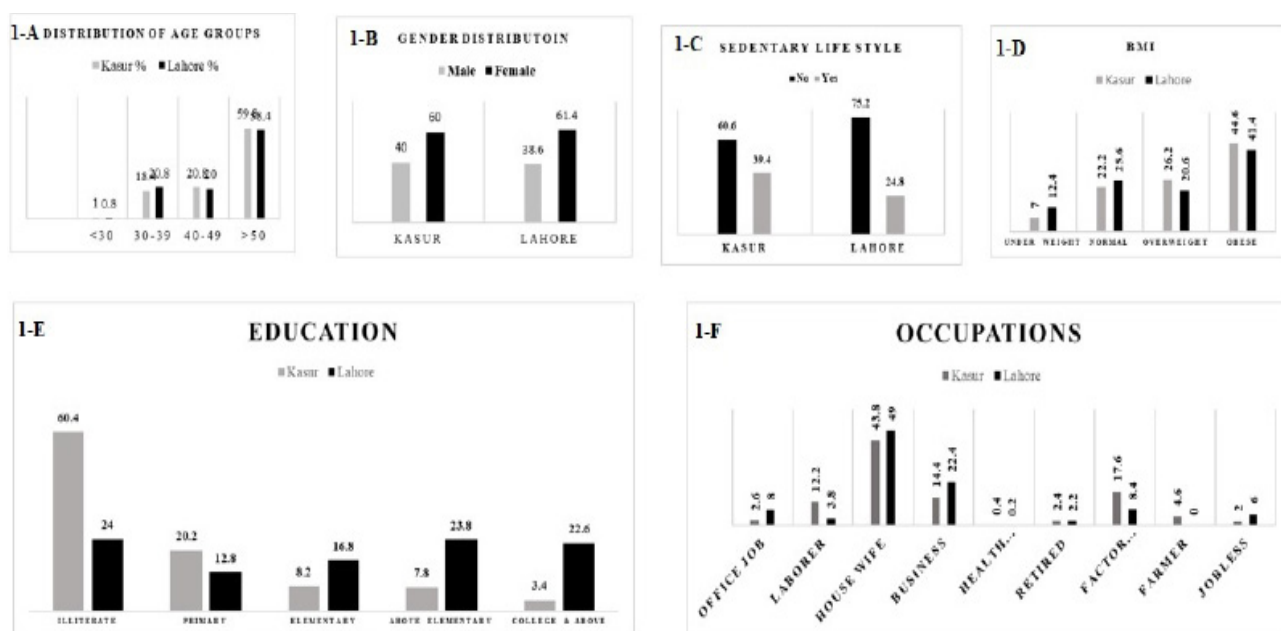

Fig 1. Comparative analysis between demographic parameters of Kasur and Lahore populations.

Table I. Sociodemographic variables and their relationship with drinking water contaminated with arsenic.

	No of individuals (%) who are exposed to water containing arsenic		χ²(p)	OR (95% CI)	p value
	≤10µg/L	>10 µg/L			
Age(years)			4.390.222)		
<30	4(44.4)	5(55.6)		1.81(0.47-6.99)	0.39
30-39	60(30.6)	136(69.4)		1.5(0.39-5.76)	0.56
40-49	71(34.8)	133(65.2)		2.07(0.55-7.79)	0.28
>50	165(27.9)	426(72.1)		1.81(0.47-6.99)	0.39
Residence near industry			386(<.001)		
No	300(57)	226(43)		NA	
Yes	0(0)	474(100)			
Smoking			35.5(<.001)	Ref	
No	240(36.1)	424(63.9)		2.6(1.89-3.59)	<0.001
Yes	60(17.9)	276(82.1)			
Occupation			77.96(<.001)		
Office Job	21(39.6)	32(60.4)		Ref	
Laborer	26(32.5)	54(67.5)		1.36(0.66-2.81)	0.40
Housewife	144(31)	320(69)		1.46(0.81-2.62)	0.21
Business	81(44)	103(56)		0.83(0.45-1.56)	0.57
Health profession	0(0)	3(100)		NA	
Retired	0(0)	23(100)		NA	
Factor worker	11(8.5)	119(91.5)		7.1(3.1-16.24)	<0.001
Farmer	0(0)	23(100)		NA	
Jobless	11(61.1)	7(38.9)		0.42(0.14-1.25)	0.12
Education			6.77(.149)		
Illiterate	113(26.8)	309(73.2)		Ref	
Primary	47(28.5)	118(71.5)		0.92(0.61-1.37)	0.68
Elementary	45(36)	80(64)		0.65(0.43-0.99)	0.05
Above elementary	48(30.4)	110(69.6)		0.84(0.56-1.25)	0.39
College and above	47(36.2)	83(63.8)		0.65(0.43-0.98)	0.04

In Kasur, 60.6% had a sedentary lifestyle, and 39.4% had an active lifestyle compared to Lahore where 75.2% of people had a sedentary lifestyle, only 24.8% had a physically active lifestyle (Fig. 1C). In Kasur, 7% were underweight, 22.2% had an ideal BMI, 26.2% were overweight, and 44.6% were obese. On the other hand, in Lahore, 12.4% were underweight, 25.6% had normal BMI, 20.6% were overweight, and 41.1% were obese (Fig. 1D).

In Kasur, 64.4% were illiterate, 20.2% got primary education, 8.2% had elementary education, 7.8% had above elementary education, and only 3.4% got college and above education. On the other hand, education status was better in Lahore. 24% were illiterate, 12.8% primary, 16.8% were elementary, 23.8% were above elementary, and 22.6% had above college education (Fig. 1E). The percentage of distribution of occupations in Kasur is 2.6% has an office job, 12.2% were labourer, 43.8% were housewives, 14.4% were businessmen, 0.4% were health professionals, 2.4% were retired, 17.6% were factory workers, 4.6% were farmers, and 2% were jobless.

Relationship of socio-demographic variables with arsenic contaminated drinking water

Table I shows association of various socio-demographic variables with arsenic contaminated drinking water. In the age categories, less than 30 (OR=1.81, P value 0.39), 30 to 39 years (OR=1.5, P value 0.56), 40 to 49 years (OR=2.07, P value 0.28) and above 50 years (OR=1.81(0.47-6.99), P value 0.39). Distribution of participants into different age groups showed no significant association. It means that arsenic concentration in drinking water equally affects all the residents of that area regardless of their age. Residences near the industrial area showed significant association between arsenic concentration with drinking water. Smoking laso had a positive association with As contaminated drinking water. Among the distribution of occupations of participants, only factory workers had a significant association.

Table II displays the link between the levels of arsenic in drinking water and blood glucose levels in the study participants. It includes the results of the χ2 test, odds ratios (OR) with 95% confidence intervals (CI), and p-values for each category of arsenic concentration. For Blood Sugar Fasting (BSF), the participants were divided into three groups based on the arsenic content in their drinking water: ≤10 µg/L and >10 µg/L. The results of the χ2 test showed a significant correlation between the arsenic content and BSF (χ2 = 142.04, p < .001). Participants in the >10 µg/L group had a higher chance of having elevated blood glucose levels compared to the reference group (≤10 µg/L) (OR = Ref).

Similarly, for Blood Sugar Random (BSR), the participants were divided into three groups based on their arsenic content. The results of the χ2 test revealed a significant association between arsenic content and BSR (χ2 = 123.31, p < .001). Participants in the highest arsenic content group (161-480 µg/L) had an increased risk of having elevated blood glucose levels compared to the reference group (120-140 µg/L) (OR = 1.34, 95% CI = 1.01-1.8, p = 0.04).

Regarding glycated haemoglobin (HbA1c), the participants were categorized into three groups based on their HbA1c levels. The results of the χ2 test showed a significant link between the arsenic content and HbA1c (χ2 = 38.85, p < .001). Participants in the highest arsenic content group (6.6-15 µg/L) had a higher probability of having elevated HbA1c levels compared to the reference group (4-5.6 µg/L) (OR = 2.17, 95% CI = 1.55-3.05, p = 0.00).

These findings reveal a significant correlation between increased arsenic concentrations in drinking water and elevated blood glucose levels, as measured by BSF, BSR, and HbA1c. The chance of elevated blood glucose levels or increased HbA1c levels were higher among individuals exposed to higher arsenic content in their drinking water than those in the reference groups. These results suggest that elevated arsenic concentrations in drinking water may contribute to developing type 2 diabetes mellitus.

Association of arsenic contaminated drinking water with blood glucose level

Table II shows the association of blood glucose level with the level of Arsenic in the drinking water.

Urinary arsenic concentration was correlated with arsenic levels in drinking water, as shown in Table III. The table lists the urinary arsenic concentration frequencies and the categories of arsenic levels in drinking water (10 g/L and >10 g/L). Arsenic levels in drinking water and urine arsenic concentration were correlated using the 2 tests. A significant correlation (r2 = 0.001) was found between the arsenic concentration in drinking water and the concentration of arsenic in the urine. All participants (100%) in the group whose drinking water arsenic level was 10 g/L had urine arsenic contents 6 g/L, demonstrating little arsenic exposure. Conversely, all subjects (100%) in the group exposed to arsenic levels in drinking water >10 g/L had urine arsenic contents above 6 g/L. These results provide strong evidence linking the amount of arsenic in drinking water to the chemical found in urine. Urinary arsenic concentrations rise in response to elevated arsenic levels in drinking water. The p-value (0.001) for this correlation is extremely small.

Arsenic level in urine samples

Table III shows association of As level in drinking water and urine samples of the subjects.

Table II. Association between drinking water's arsenic content and blood glucose levels.

Blood glucose level	Number of subject (%) exposed to arsenic in drinking water		χ²(p)	OR (95%CI)	p-value
	≤10 µg/L	>10 µg/L			
BSF			142.04(<.001)		
80-100	150(35.9)	268(64.1)		Ref	
101-125	0(0)	82(100)		NA	
126-165	150(30)	350(70)		1.31(0.99-1.72)	0.06
BSR			123.31(<.001)		
120-140	150(42.1)	206(57.9)		Ref	
141-160	0(0)	152(100)		NA	
161-480	150(35.1)	277(64.9)		1.34(1.01-1.8)	0.04
HbA1c			38.85(<.001)		
4-5.6	150(32.9)	306(67.1)		Ref	
5.7-6.5	88(42.5)	119(57.5)		0.66(0.47-0.93)	0.02
6.6-15	62(18.4)	275(81.6)		2.17(1.55-3.05)	0.00

BSF defined as Blood Sugar Fasting; BSR, Blood sugar Random; HBA1c, glycated hemoglobin.

Table III. Association between level of Arsenic in drinking water and urinary arsenic.

	Arsenic level in drinking water		χ^2(p)
Arsenic in urine	≤10 µg/L	>10 µg/L	<.001
<6	300(100)	0(0)	
6-30	0(0)	244(100)	
30-85	0(0)	356(100)	
85-96	0(0)	63(100)	

Table showing association between arsenic level in drinking water below 10 µg/L and above 10 µg/L and their association with arsenic in urine.

Table IV compares the median (interquartile range) levels of arsenic, glucose (BSF, BSR), and urine arsenic concentrations in people from Kasur and Lahore, separated by whether they had diabetes. The table also includes the p-values, which show whether the differences between the groups are statistically significant. Differences between Kasur and Lahore participants with and without diabetes were first observed in their glucose levels. Median BSF levels in Kasur were 97 mg/dL (IQR 91-106) for people with diabetes and 141 mg/dL (IQR 134.75-151) for those without diabetes (p 0.001). The results for both BSR and HbA1c were consistent with one another. There was a statistically significant difference between the arsenic levels in drinking water in Kasur and Lahore (p = 0.003). Arsenic levels in Lahore's water supply were 80 g/L (interquartile range), while in Kasur, they were 72 g/L (IQR: 38-103) (IQR: 70-105). Arsenic levels in drinking water were compared to the prevalence of diabetes. However, there was no statistically significant correlation found.

Diabetic subjects in both Kasur and Lahore exhibited higher median values for urine arsenic concentrations than healthy controls. Median urine arsenic concentrations in Kasur were 44.5 g/L (IQR: 16-73) in people without diabetes and 51 g/L (IQR: 35-95) in people with diabetes (p 0.001). In a similar vein, people without diabetes in

Lahore had a median urine arsenic content of 22 g/L (IQR: 0.68-29) compared to 25 g/L (IQR: 3.8-32.25) among those with diabetes (p = 0.005). These results demonstrate statistically significant correlations between diabetes and both glucose and arsenic levels in the urine. Arsenic levels in drinking water were not linked to an increased risk of diabetes. Arsenic levels in drinking water were measured in both Kasur and Lahore, and the results showed significant discrepancies between the two cities.

DISCUSSION

A study was conducted in Kasur and Lahore, Pakistan, to investigate the association between arsenic and type 2 diabetes. We reported in our previous study that many heavy metals are causing groundwater contamination. As a whole, environmental pollutants are diabetogenic (Shokat *et al.*, 2022).

It is important to consider gender when interaction with the environment is studied. We could not represent the experiences of the other group based on the results we found with one group. Research should therefore involve both men and women. For this study, we collected data of both males and females to compare how exposure to arsenic affects both genders (Chubb and Derrik, 2020). Health is affected adversely if a person is exposed to arsenic at high levels. Diabetes, cardiovascular disease, and skin lesions are all possible outcomes of arsenic exposure. Brain growth is also negatively affected, and death can result in early age exposure (WHO, 2022).

Researchers found that non-accidental deaths are associated with populations with naturally high levels of arsenic and drinking water (Nuvolone *et al.*, 2023). Arsenic concentration is higher in older people due to exposure to arsenic for a prolonged period (Abbas and Cheema, 2019). On the other hand, in our research, age didn't show any association with arsenic concentration in drinking water.

Table IV. Comparison of relationship of Median (IQR) level of arsenic in drinking water, urinary samples and bold glucose levels in the subjects of Kasur and Lahore.

	Kasur			Lahore		
	Non-diabetic	Diabetic	P	Non-diabetic	Diabetic	P
BSF	97(91-106)	141(134.75-151)	<.001	90(85-95)	135(130-139.25)	<.001
BSR	145(131-153)	280.5(232-353.5)	<.001	128(124-134)	259.5(229-287)	<.001
Hb1AC	5.1(4.6-5.5)	8(7-9)	<.001	4.8(4.3-5.3)	6.6(6.3-7.23)	<.001
Arsenic in drinking water (g/L)	72(38-103)	80(70-105)	0.003	50(1.7-56)	50(7.4-56)	0.065
Arsenic in urine (g/L)	44.5(16-73)	51(35-95)	<.001	22(0.68-29)	25(3.8-32.25)	0.005

BSF defined as blood sugar fasting; BSR, blood sugar random; HB1AC, glycated hemoglobin.

Socioeconomic status poses a significant risk factor for diseases caused by environmental factors (Eick *et al*., 2019). In our research data, many participants were illiterate, and a few were elementary and above elementary. At the same time, only a few participants were above college. Resisting near arsenic exploitation sites (volcanic, mining and other industrial activities) can affect various human body organs (Bundschuh *et al*., 2021). People's health living close to the industrial area was most affected by contaminants in groundwater. In our findings, residence near industrial areas positively correlates with arsenic exposure.

Arsenic's adverse effects can be exacerbated by smoking (Wei *et al*., 2021). In our findings, smoking has a significant association with arsenic exposure. Working environments (including mining and smelting) cause an increase in arsenic concentration in urine, hair and blood (Zhao *et al*., 2023). Similarly, in our data, smoking has an association with arsenic concentration. Heavy metals (lead, nickel and arsenic) were reported in the blood of factory workers (Okpogba *et al*., 2019). Factory workers reported a relative concentration of arsenic in their hair (Javed *et al*., 2022). The concentration of arsenic was significantly high in printing factory workers (Sirinara *et al*., 2023). Occupations directly affect total arsenic intake. In our data among the different types of occupations, factory workers have a significant association with arsenic in water used for drinking and urine.

The type of water used for drinking is an independent variable that affects arsenic concentration in human blood and urine. In rural areas, the population relies mainly on tube-wells for drinking water; this was exposed to arsenic. Arsenic is found in groundwater, tube wells, and tap water in exposed areas with drinking water above 10 g/L (Murtaza *et al*., 2020). It was observed that people who consume filtered water are less exposed than those who consume tap water. Many Participants were found to drink tap water (Ahmad and Khan, 2023). A positive correlation was observed between arsenic concentration in drinking water and urinary arsenic (Takayama *et al*., 2021). Diabetes and arsenic are fatal to human health. The combination of both can produce drastic results. Diabetes contributes to a large number of deaths globally. Similarly, arsenic is the 9th number in causes of death (Lin *et al*., 2020).

Several lifestyle factors contribute to type 2 diabetes, including obesity, residence, physical inactivity, type of drinking water, working environment, and smoking. But it can be managed by making some modifications to lifestyle. In the present study, we find a positive association with arsenic present in water used for drinking towards type 2 diabetes (Szczechla *et al*., 2023).

This study found A positive association between arsenic exposure and insulin resistance. Arsenic exposure increases fasting blood glucose levels and hyperglycemia risk (Mondal *et al*., 2020). There is a higher level of arsenic in water samples from district Kasur than in district Lahore. The study confirms that 97% of the water samples from Ibrahim Abad and Badarpur had high levels of arsenic concentration (58-3800 μg/L), and rural areas of district Kasur are unsafe to drink. An extensive range (Arshad and Imran, 2017; Ahmad *et al*., 2013). Blood glucose levels increase with the increase of air pollutants, which induces oxidative stress. In Kasur, the mean arsenic was high in water used for drinking, which resulted in a rise in blood sugar random, blood sugar fasting and Hb1Ac. When we compare the level of arsenic in water used for drinking in Kasur and Lahore, the mean of arsenic in drinking water was high in Kasur as compared to Lahore; similarly, blood glucose levels were also high in Kasur (Li *et al*., 2019).

Comprehensive awareness efforts are needed to educate the public about drinking water contamination and health risks. These programs should help people make water-use decisions. One option is workshops and lectures with specialists on drinking water contamination's sources, causes, and health effects. These seminars should encourage questions and conversation. Brochures, leaflets, and posters illustrate the consequences of contamination and the significance of safe water usage. Reaching more people requires working with local health agencies (Artiga and Hinton, 2019). These organizations can help integrate awareness programmes into health campaigns, health fairs, and community outreach. TV, radio, newspapers, and social media can also create awareness. Articles, interviews, and films can assist in spreading critical information.

Interactive activities, demonstrations, and debates can teach kids about pollution and safe water habits. Community leaders, influencers, and local groups can boost awareness programmes. Their support can build public trust and engagement (Baldrighi *et al*., 2022). Safe drinking water requires public solutions. To protect oneself, teach them about water filters, boiling water, and bottled water. Guide recognizing contamination and reporting water quality issues. Regular arsenic testing of drinking water is essential. Publicizing testing services and understanding test findings can help people choose water sources (Podgorski and Berg, 2020). Community-led water quality monitoring promotes responsibility and collaboration. Awareness campaigns must be evaluated and improved. Effectiveness, public feedback, and strategy adjustments ensure continuing progress and handle changing problems (WHO, 2020).

CONCLUSION

Arsenic can play a role as a carcinogenic and non-carcinogenic agent. Its ability to destroy DNA makes it

more terrific. Arsenic metabolites determine its mode of action as carcinogenic (different types of cancers) or non-carcinogenic (CVD, hypertension and diabetes). In our observation, the areas with high arsenic concentrations showed increased blood glucose levels in the participants. Living near industrial areas, smoking, and drinking tap water (demographic factors which can be affected by arsenic concentration) showed significant association. It was observed that Kasur was more exposed to arsenic as compared to Lahore. It was concluded that areas with high arsenic concentrations in drinking water showed evidence of high urinary arsenic. Based on all investigation, it was concluded that arsenic was a diabetogenic agent.

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Phytochemical Analysis of Seven Medicinal Plants and Evaluation of Anti-Oxidant and Anti-Diabetic Activities

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ABSTRACT

According to the report of world health organization, 80% population utilized medicinal flora to treat various diseases. *Citrullus colocynthis, Allium sativum, Momordica charantia, Zingiber officinale, Cinnamomum verum, Eugenia jambolana* and *Moringa oleifera* trees have a wide range of therapeutic as well as nutritional benefits. Specific phytochemical components found in plants which act on metabolic activities as well as best antioxidants. By scavenging free radicals like hydro peroxide and peroxides an antioxidant slows down or prevents oxidative damage to organisms' cells, lowering the risk of degenerative illnesses. Whereas, majority of plant extracts control diabetes by inhibiting alpha amylase. This study evaluates the phytochemical analysis of seven medicinal plants and evaluation of anti-oxidant and antidiabetic activities by qualitative phytochemical analysis of plant extracts by preparing plants extract in ethanol. Antioxidant activity was performed by the determination of total phenolic and total flavonoids content. Similarly, α-amylase inhibitory activity was assessed by decrease in maltose. A verified dinitro salicylic acid technique was used to compute the maltose equivalent. Phytochemical screening of plants showed the presence of alkaloids, flavonoids, saponins, sterols, phenols and tannins. Whereas, the plant extracts of garlic, and bitter apple exhibited strong antioxidant activity. The plant extract of cinnamon sticks, bitter gourd and blackberry showed excellent inhibition of α amylase enzyme. So this study strongly suggested the use of natural flora, fruits and herbs to cure various illnesses.

INTRODUCTION

Medicinal plants have been utilized from years, and many civilizations yet depend on them for their basic medical requirements. Today, it is widely acknowledged that the foundation for a variety of important human health, social, and economic support systems and various advantages lies in the herbal medicines. These compounds contribute with various ways and are used to cure different illnesses, which include life-threatening conditions like HIV-AIDS, cancer, and diabetes (Street and Priciploo, 2013). It is currently known that plants that normally produce and store certain secondary metabolites and vitamin have

therapeutic effects (Huzaifa *et al*., 2014).

The World Health Organization (WHO) estimates that about three-quarters of the population uses herbs in the form of traditional medicines to manage illnesses. Particularly 80% of people in rural areas or in poor nations trust traditional medicine as a kind of treatment. Medicinal flora are used in various pharmaceutical medicines to treat diseases in about 74% of cases (Salmerón-Manzano *et al*., 2020). Out of the 21,000 species of therapeutic plants that are registered by the WHO globally, 2500 plant species are known as medicinal plants. Antimicrobial drugs are used to treat infectious disorders in modern era. Owing to the imperceptible use of antimicrobial medications, frequent medication tolerance develops, which becomes a medical issue associated with globalization (Hegde *et al*., 2011).

According to the WHO report, 80% population utilized medicinal flora to treat various diseases in under developed countries. *Citrullus colocynthis* (bitter apple), *Allium sativum* (garlic), *Momordica charantia* (bitter gourd), *Zingiber officinale* (ginger), *Cinnamomum verum* (cinnamon), *Eugenia jambolana* Lam. (blackberry) and *Moringa oleifera* trees have a wide range of therapeutic as well as nutritional benefits. The majority of plant parts,

including leaves, seeds, fruits, roots, stem, and bark, are used as diets or remedies in number of nations, with particular emphasis on traditional societies (Salmerón-Manzano *et al.*, 2020). Different phytochemicals, including phenolic compounds, alkaloids, flavonoids, lignin, tannins and glycosides are naturally found in plants and plant-based products. The much more prevalent phytoconstituents in variety of vegetables, fruits and medicinal plants that have antioxidant properties are phenols and flavonoids. Natural antioxidants like flavonoids and phenols are becoming more and more well-liked these days, because of the possible toxicological consequences of synthetic antioxidants. By scavenging free radicals like hydro peroxide and peroxides an antioxidant slows down or prevents oxidative damage to organisms' cells, lowering the risk of degenerative illnesses. Numerous serious human illnesses, including Alzheimer's disease, cancer, heart, fibrosis, atherosclerosis, renal, and liver conditions, neurological disorders, ageing and arthritis may be brought on by abnormal free radical generation. The antioxidant and other biological actions of a number of medicinal plants have been investigated in research (Aryal *et al.*, 2019).

Whereas, diabetes mellitus causes abnormalities in insulin secretion or function, which alters the equilibrium of lipid and glucose metabolism. It is a serious metabolic disorder with a high death rate and substantial healthcare expenses. According to rough estimates, 387 million persons worldwide have diabetes, which resulted in 4.9 million fatalities in year 2014. Diabetes mellitus is a condition in which blood glucose levels stay high after eating, so this factor must be taken into consideration in diabetes management. Medications are costly as well, given that roughly 80% of diabetics belong to middle income nations. Majority of plant extracts control diabetes by inhibiting alpha amylase (Jemaa *et al.*, 2017).

MATERIALS AND METHODS

Plant collection, extraction, and fractionation
The Lahore local market was used to obtain the botanical plants and flower supplies of C. *zeylanicum, A. sativum, M. oleifera* and *C. colocynthis*. All of the plant and fruit extracts were verified and identified by the Department of Botany at Punjab University Lahore and a voucher specimen was kept there. The plant parts and fruits were then divided, cleaned, and chopped into little pieces before being air dried at room temperature in a dry, open area. In order to achieve a uniform weight, additional plant and fruit components were dried in an oven at a controlled temperature of 40 ºC. Whatman No. 1 filter paper was used to purify the resulting supernatant. These were then put through a grinder to get a coarse powder.

Determination of biochemical components
Alkaloids
When 2.75ml of extract and 2.75ml of Wagner's reagent and 2.75 of iodine and 6.25 of potassium iodide in 100ml were combined, a brownish precipitate indicated the presence of alkaloids.

Terpenoides
By mixing 2.75ml of cinnamon extract with 2.75ml of chloroform and then added conc. sulphuric acid, a deep reddish brown color indicates the presence of terpenoids.

Anthraquinones
2 ml of extract was cooked with 10% HCl for a brief period of time in a water bath. It was filtered and then chilled. The filtrate was then mixed with an equal volume of $CHCl_3$ and few drops of 10% ammonia then heated. So the production of rose pink color is a sign that anthraquinones are present.

Tannins
Okerulo and Harbone ferric chloride test was used to confirm tannins presence in the samples. 1.5ml of the extract was mixed with 3 drops of a 5% ferric chloride solution, and the resulting color was observed. A precipitate which is dark greenish indicated tannins presence.

Saponin
The Harbone method was used to determine whether saponins are present in the samples. A beaker containing one gram of sample and 20 ml of water is heated to boil for 4 min and allow the solution to cool.

Flavonoids
Harbone procedure is use to determine the presence of flavonoids in the sample. 2.5 g of the material was cooked for 8–10 min in 20 ml of distilled water before being filtered. The filtrate was acidified with 5-7 drops of dil. HCl and left to stand. A yellow color is shows after adding 5.5ml of the filtrate aliquots and 2ml of 10% NaOH, indicating the potential existence of flavonoids.

Glycoside
11 ml of 50% H_2SO_4 is added with 1.5 ml of the extract and then mixture is heated for 16 min in boiling water bath and then add 10ml of Fehling's solution and again heat the mixture. There is a brick-red precipitate formed which confirm glycosides.

Cardiac glycoside
In 2.5 ml of plant extract, 4.5% $FeCl_3$ is added and the mixture is let to stand for 2 min. The test tube's corner is protected to contact the top surface while adding 2ml of

Conc. H_2SO_4. The existence of 2-deoxy sugars containing cardiac glycosides was identified by the color changing from green to blue.

Sterols

A total of 3 milliliters' of concentrated sulfuric acid is mix with the plant extract by following Solihah technique. Sterols are present as confirmed by the red precipitate's formatio

Phytosterols detection

Plant extract is mix with a few drops of sulfuric acid, and the filter then allowed to stand after vigorous shaking by following Salkowski technique. Golden yellow hue development indicates the presence of triterpenes.

Total phenolic content assessment

The total phenolic contents from the extract of *A. sativum, C. zeylacium, M. oleifera* and *C. colocynthis* were processed using the Folin-Ciocalteu colorimetric technique. Mix appropriate amount of plant extracts with 20% of sodium carbonate solution and reach the volume of 25mL to get a standard gallic acid solution. Several strengths of gallic acid solutions in methanol were made from the reference solution.

The absorbance at 760 nm was measured in comparison to a control. When phenols from extracts were oxidized a dark blue color results, which may be measured with a UV-visible spectrophotometer. All experiments were performed in triplicate by using the average absorbance results, calibration curves were produced for various gallic acid concentrations.

Samples were taken in triplicate for each assay, and the total phenolic content of the extract was analyzed by drawing a calibration curve by using average absorbance values. The gallic acid equivalents per gram of dry weight of the sample served as a measure of the overall phenolic content of the extracts. Using the formula, the total phenolic content of all samples were calculated.

$$C = c\frac{V}{m}$$

where V is extract volume in mL, m is extract mass in g, C is total phenolic content mg GAE/g dry extract, c is concentration of gallic acid determined from calibration curve in mg/mL.

Total flavonoids content assessment

The total amount of flavonoids in the extracts were determined using a colorimetric assay. Mix appropriate amount of plant extracts with 10% of $AlCl_3$ solution and potassium acetate solution of about 1.0 M, then reach the volume of 25mL and take absorbance values at 415 nm by the help of spectrophotometer. With the aid of a calibration curve, a linear equation was employed to express the total flavonoids concentration as quercetin equivalents. Readings were taken in triplicate and the average absorbance value was used to determine the total flavonoid content. The flavonoid content was reported as quercetin equivalent (mg QE/g) and a linear equation was applied based on the standard calibration curve.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

Using a modified method of Nithianantham's DPPH free radical scavenging test, the antioxidant activities of the extracts were assessed *in vitro*. The quickest and most verified method for calculating antioxidant is scavenging potential. Because of electron reduction, DPPH in methanol undergoes oxidation to generate a deeper violet color and the resulting color then transforms from deep violet to yellow. However, because of its significant absorption at 517 nm, deep violet color was visible in DPPH solution. The capacity of a test sample to neutralize DPPH free radicals reveals its antioxidant or free radical scavenging potential, which illustrates its effectiveness, interception, prevention, and repair mechanisms in biology.

Prepare a solution of DPPH (0.1 M) in a volumetric flask by adding 0.001 grams of DPPH and diluting it with methanol to a final volume of 25 milliliters. As a result, DPPH free radical solution was created and showed purple in color. It was then kept at 20°C for future usage.

The sample solutions were mixed with 3 mL of DPPH solution and left at room temperature for 30 min while maintained in the dark. A control solution was created by mixing 3 mL of DPPH solution with 2 mL of methanol. Then, a spectrophotometer was used to detect the absorbance at 517 nm. Ascorbic acid was used to obtain standard values. A graph of concentration versus percentage inhibition was used to determine the 50% inhibitory concentrations (IC_{50} values) of the extracts.

The sample concentration required to scavenge 50% of the DPPH free radical is known as the IC_{50} value. Three different readings were taken. The concentration at which the radical scavenging capacity of the extracts is 50% was indicated by its IC_{50} value. The IC_{50} of the extract and the standards were calculated using a graphical depiction. The percentage of inhibition was calculated using the formula shown below:

Antioxidant activity (%) = ((OD blank - OD sample)/ OD blank) × 100

Amylase β assay

For maltose calibration curve prepare 10 labelled tubes with maltose dilutions ranging from 0.4 to 55 micro-

moles per ml. Pipette 2 ml of every maltose dilution into a line of tubes that have been labelled. Prepare DNS, starch and enzymes solution to perform the tests. Add 2 ml of the dinitrosalicylic acid colorant. cooled to room temperature after 6 min in a boiling hot water bath. Each tube should contain 10 mL of reagent-grade water. Read A540. Maltose micromoles versus the A540 plot.

In-vitro *anti-diabetic activity*
     α-amylase inhibitory activity
     The inhibition of enzyme was calculated using the quantity of reducing sugar (maltose equivalent) released throughout the experiment by following modified DNS technique. Initially, 2 mL of the extract was pre-incubated for 32 min with α-amylase 1 U/mL. (Pipette 0.6 mL of corresponding enzyme with various concentrations into a sequence of labeled test tubes. A blank with 0.6 mL of water suitable for reagents was also included. Incubate tubes at 25°C for 4–5 min to achieve temperature equilibration. At certain intervals, add 0.6 mL of the starch solution. Add 2 ml of the Dinitrosalicylic acid color reagent to each tube at predetermined intervals after 4 min of incubation. All tubes should be incubated for 5 min in a hot water bath. Before adding 10 mL of reagent grade water, let the mixture reach room temperature. After thoroughly blending, compare A540 to blank. Before incorporating 2 mL of a 1% w/v starch solution, determine the micromoles of maltose liberated from the standard curve. The solution was then incubated for an additional 10 min at 37°C. Afterward, 2 mL of DNS reagent was added to stop the reaction.

     The mixture was heated for five min. Amylase was omitted from one blank, and plant extracts were absent from another. These blanks were both replaced with equal amounts of buffer (20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 20 °C). 540 nm was used as the wavelength to measure the absorbance. Using a standardized graph and amaryl and glucophage as a positive control, the reducing sugar produced by starch as maltose equivalent was estimated. The anti-diabetic activity was determined using the following equations using the % inhibition of alpha-amylase:

     % reaction = (maltose) test / (maltose) control x 100

RESULTS

Phytochemical analysis of medicinal plants
     The presence of bioactive chemical components in plants is responsible for their pharmacological actions. Table I shows that ethanol extract of plants contains all of the components which are tested except those which are absent. Table I shows the qualitative analyses of medicinal plants.

Antioxidant activity
     In anti-oxidant activity, the ethanolic extract of *A. sativum, C. zeylanicum, C. collocynthis* and *M. oleifera* at concentration of 0.7mg/mL shows significant results of of 4.4mg/mL, 3.72mg/mL, 3.9mg/mL, 3.6mg/mL, 2.4mg/mL, 2.3mg/ as compared to standard and control of gallic acid. In other respective extract concentrations of 0.6mg/mL and 0.5mg/mL, TPC is 4.2 mg/mL, 3.7 mg/mL, 3.45 mg/mL, 3.4 mg/mL, 2.30 mg/mL and 2.2mg/mL while at 0.5mg/mL is 4.1mg/mL, 3.21mg/mL, 3.2mg/mL, 3.2mg/mL, 2.31mg/mL and 2.0mg/mL of *M. oleifera, C. zeyacinum, C. colocynthis* and *A. sativum*. Maximum TPC of plant extract shows in synergistic effect at 0.7mg/mL concentration of *Moringa* and *Rubus subg. Rubus, C. zeylanicum* and *A. sativum* and *C. zeylanicum* and *M. charantia* that is 5.21mg/mL, 4.55mg/mL and 4.4mg/mL as compared to standard and control, respectively. In other respective concentrations 0.6mg/mL, and 0.5mg/mL TPC is 5.11mg/mL, 4.53mg/mL, 4.3mg/mL, 5mg/mL, 4.5mg/mL and 4mg/mL. Total phenolic content of plant extracts in mg GAE/g dry extract wt. is shown in (Table II).

Table I. Phytochemical analysis of some medicinal plants.

S. No	Phytochemicals	Distilled water/ethanol	*C. verum*	*A. sativum*	*C. colocynthis*	*M. olefeira*
1	Alkaloids	+	+	+	+	+
2	Cardiac glycosides	+	+	+	-	-
3	Flavonoids	+	-	+	+	+
4	Tannins	+	+	+	+	+
5	Terpenoides	+	+	-	-	-
6	Anthraquinones	+	+	-	-	-
7	Glycosides	+	+	+	-	-
8	Saponins	+	-	-	+	+
9	Sterols	+	-	-	-	+

C. verum, Cinnamomum verum; *A. sativum, Allium sativum*; *C. colocynthis, Citrullus colocynthis*; *Moringa oleifera, Moringa oleifera.*

Table II. Total phenolic (TPC) and total flavonoids content (TFC) of selected plants.

TPC & TFC	*A. sativum* (n=3)	*C. zeylanicum* (n=3)	*C. colocynthis* (n=3)	*M. oleifera* (n=3)
TPC	3.4±0.2	3.6±0.36	3.46±0.25	4.23±0.15
TFC	0.41±0.01	0.63±0.01	0.72±0.01	0.95±0.01

In anti-oxidant activity of total flavonoids, the ethanolic extract of *C. collocynthis, M.* oleifera, *C. zeylanicum* and *A. sativum* at concentration of 0.7mg/mL shows significant results of 1.92mg/mL, 1.07mg/mL, 0.97mg/mL and 0.85mg/mL at 0.65mg/mL and 0.41mg/mL as compared to standard and control of quercetin. In other respective extract concentrations of 0.6mg/mL and 0.5mg/mL, TFC is 1.93 mg/mL, 1.04 mg/mL, 0.95 mg/mL, 0.83 mg/mL, 0.63 mg/mL and 0.40 mg/mL while at 0.5mg/mL is 1.94 mg/mL, 1.02 mg/mL, 0.94 mg/mL, 0.82 mg/mL, 0.62 mg/mL and 0.42 mg/mL of *C. collocynthis, M.* oleifera, *C. zeylanicum* and *A. sativum* (Table II). Maximum TFC of plant extract shows in synergistic effect at 0.7mg/mL concentration of *Moringa* and *Rubus subg. Rubus, C. zeylanicum* and *A. sativum* and *C. zeylanicum* and *M. charantia* and 1.89mg/mL, 1.33 mg/mL and 1.22 mg/mL as compared to standard and control, respectively. In other respective concentrations 0.6mg/mL, and 0.5mg/mL TFC is 1.87mg/mL, 1.32 mg/mL, 1.25 mg/mL, 1.88 mg/mL, 1.3 mg/mL and 1.24 mg/mL. Total flavonoids content of plant extracts in mg GAE/g dry extract wt. is shown in (Table III, Fig. 1).

Table III. Synergistic effect of TPC and TFC of selected plants (n = 3)

Plant sample	TPC (mg GAE/g dry extract wt)	TFC (mg QE/g dry extract wt)
Moringa and *Rubus* subg. *Rubus*	5.10±0.10	1.88±0.01
C. zeylanicum and *A. sativum*	4.52±0.02	1.31±0.01
C. zeylanicum and *M. charantia*	4.23±0.20	1.23±0.01

DPPH scavenging activity

Anti-oxidant activity of plant extract is to measure the DPPH radical scavenging effect at different concentrations. Percentage inhibition and IC_{50} of DPPH radicals of *A. sativum, C. zeylanicum, C. colocynthis* and *M. oleifera* is 0.5989 mg/mL, 0.5788 mg/mL, 0.6034 mg/mL, 0.6179 mg/mL, 0.6193 mg/mL and 0.6118 mg/mL. The ethanol extract of *M. oleifera* shows higher radical scavenging activity than remaining extracts. While maximum percentage inhibition and IC_{50} values shows by synergistic effect of *C. zeylanicum* and *A. sativum, Moringa* and *Rubus subg. Rubus* and *C. zeylanicum* and *M. charantia* that is, 0.6108

mg/mL, 0.5992 mg/mL and 0.6029 mg/mL (Table IV).

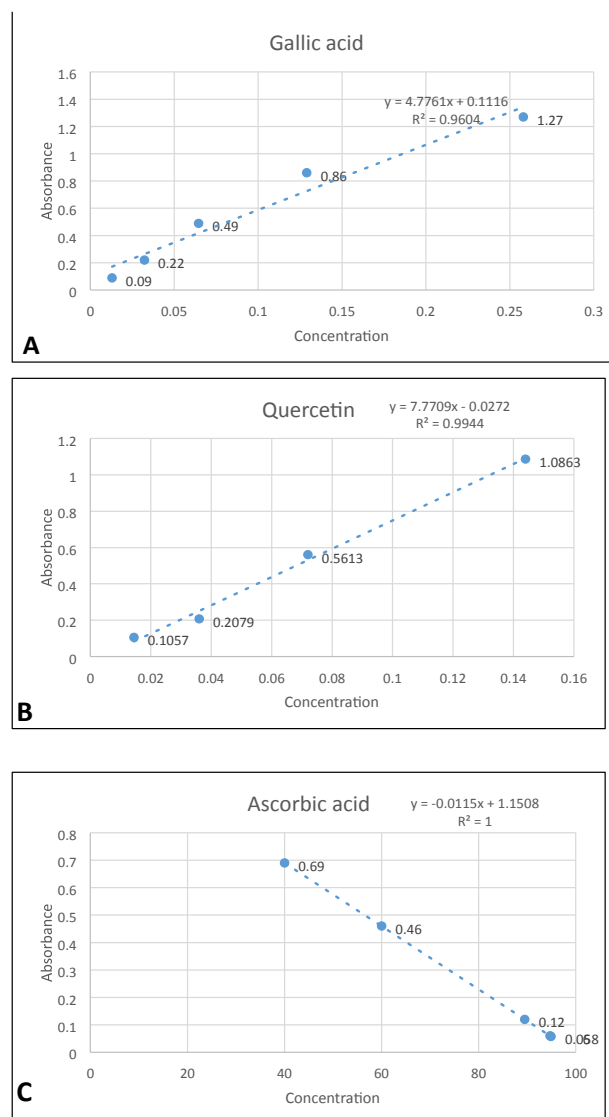

Fig. 1. Graphical representation of antioxidant activity.

Table IV. IC_{50} values of extract at different concentrations.

	IC_{50} (mg/mL)
A. sativum	0.5989
C. zeylanicum	0.5788
C. colocynthis	0.6034
M. oleifera	0.6118
C. zeylanicum and *A. sativum*	0.6108
Moringa and *Rubus subg. Rubus*	0.5992
C. zeylanicum and *M. charantia*	0.6029

In the present study, maximum synergistic antioxidant activity of DPPH is find from *C. zeylanicum* and *A. sativum* extract as compared to the standard ascorbic acid.

Anti-diabetic activity

This is indicated α-amylase inhibitory activity of plant extracts. The ethanol extracts of *C. verum, M. charantia, Rubus s.ubg. Rubus, C. colocynthis* seeds*, C. colocynthis* pulp*, Z. officinale, M. oleifera* and *A. sativum* shows appreciable α-amylase inhibitory activity of 91%, 83%, 77%, 66%, 58%, 58%, 50% and 50%, respectively, when compared with amaryl and glucophage 91% and 77% (Table V, Fig. 2).

Table V. α-amylase inhibitory activity of plant extracts. Alpha Amylase = 1U/mL.

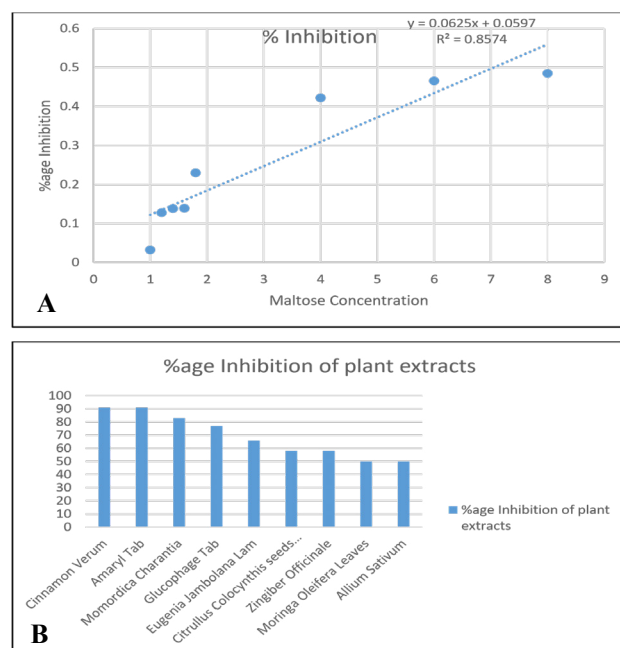| Concentration (1000mg/mL) of plant extracts	%age Inhibition
C. Verum	91
Amaryl	91
M. Charantia	83
Glucophage	77
E. Jambolana Lam.	66
C. colocynthis seeds and pulp	58
Z. officinale	58
M. oleifera leaves	50
A. sativum	50

Fig. 2. Graphical representation of anti-diabetic activity.

DISCUSSION

All medicinal plants help to protect humans against various illnesses due to their biologically active ingredients. Medicinal plants are rich in nutrition (Singh and Kumar, 2017). According to the research, therapeutic plant extracts mentioned above include a number of phytonutrients. Phytonutrients provide plants with flavor, color and fragrances. They also function as a plant's natural defense mechanism and shield it from infections, parasites, and herbivorous insects and animals. These therapeutic plant extracts contained saponin, flavonoid, tannin, reducing sugar, steroid, glycosides, anthraquinones, cardiac glycosides, sterols, and terpenoid, according to this study (Ali and Ibrahim, 2019).

Several phytonutrients, such as carotenoids and flavonoids have antioxidant properties and lower the chance of numerous illnesses. Many illnesses and the aging processes have been linked to reactive oxygen species. These free radicals are often produced by oxidative metabolism, inflammation, and oxidative degradation of lipids and induce tissue damage via increasing level of per oxidants. Harmful consequences of reactive oxygen species are reduced by antioxidants. Plants' therapeutic efficacy is derived from biologically active phytonutrients that have a clear psychical impact on the human body. In our study of *A. sativum, C. colocynthis, Z. officinale, M. charantia, C. verum, M. charantia, E. Jambolana* and *M. oleifera* the examination of phytonutrients on the aqueous and ethanolic extricates shows the enriched amount of metabolites such as flavonoids, alkaloids, cardiac glycosides tannin, glycosides, phenolic compounds, saponins, steroids, terpenoids and anthraquinones (Singh and Kumar, 2017).

Flavonoids found in *A. sativum, C. colocynthis*, and *M. oleifera* might be utilized to cure diseases such as toothache, common cold, diarrhea, edema, fever, and dental caries. Saponins/steroid or triterpenoid glycosides have foaming capabilities, bitter or astringent flavor and hemolytic impact on red blood cells. Saponins have both positive (reducing cholesterol) and harmful (cytotoxic permeabilization of the gut) qualities, as well as structure-dependent biological actions. Tannins may have antibacterial activity by proteolytic enzymes, causing cell membrane lysis, interfering with microbial adhesions and inhibiting protein synthesis. They have also been shown to have physiological benefits such as anti-secretolytic, anti-irritant and anti-parasitic properties (Singh and Kumar, 2017). *C. colocynthis, A. sativum, M. charantia, Z. officinale, C. verum, E. jambolana,* and *M. oleifera* extracts included phenolic components, flavonoids compounds, and free radical scavengers (Singh and Kumar, 2017).

Alpha amylase activity in the small intestine correlates with an increase in postprandial glucose levels, inhibiting is significant in the treatment of type II diabetes mellitus. Flavonoids are believed to possess alpha amylase inhibitory capability, and their effectiveness is similar to that of diabetes medications. Several medicinal plants may have alpha amylase inhibitory properties owing to sulfur-containing chemicals. The minimum Inhibitory concentration value of (IC_{50}) these medicinal plants shows that they are more powerful than the conventional drugs Amaryl and Glucophage (Ahmed *et al*., 2020).

CONCLUSION AND RECOMMENDATIONS

This study demonstrates that aqueous and ethanolic extracts of *C. colocynthis, A. sativum, M. charantia, Z. officinale, C. verum, E. jambolana* and *M. oleifera* typically contain active phytoconstituents, which promote numerous therapeutic usage contributed to them in folk remedies. Even though their different roles in this research are not evaluated, it has been revealed that the most prevalent bioactive components in plants are commonly flavonoids and tannins alkaloids, glycosides, tannins, saponins, sterols, and cardiac glycosides. Flavonoids in specific and phenolic compounds in particular, now have capacity to defend against oxidative stress. Thereby, in this research, the existence of flavonoids and phenolic compounds in the extracts could be attributed to the antioxidant ability.

C. verum, M. charantia, E. jambolana, C. colocynthis, Z. officinale, M. oleifera and *A. sativum* possesses alpha amylase inhibitors that compete with one another. While *M. oleifera* and *A. sativum* are less effective amylase inhibitors and *C. verum, M. charantia, E. jambolana, C. colocynthis* and *Z. officinale* are more effective inhibitors. Despite the fact that all medicinal herbs can be utilized to treat type 2 diabetes mellitus.

Statement of conflict of interest
The authors have declared no conflict of interest.

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CITATIONS

Prof. Dr. Muzaffer Ahmad Lifetime Achievement Award 2023

Prof. Dr. Abdullah G. Arijo
Visiting Faculty
Shaheed Benazir Bhutto University of,
Veterinary & Animal Sciences,
Sakrand, Sindh

rof. Dr. Abdullah G Arijo was born in 1962 at district Larkana. He graduated in biological sciences from the University of Sindh, Jamshoro and did MSc in Zoology with specialization in Parasitology from the University of Karachi. In 1993, Dr. Arijo was granted World Bank Award for higher studies and obtained a Ph.D. from the University of North Wales, the Bangor-United Kingdom in 1997. His teaching carrier started as lecturer in Pakistan Air Force College Base Masroor, Karachi in 1986. In 1988 he shifted to Sindh Agriculture University Tandojam as Lecturer.

Dr. Arijo is an active scientist, has presented his research in conferences held in various countries around the world. He is an active member of many scientific societies including British Society for Parasitologists, Society of Biological Sciences, North Wales, Zoological Society of Pakistan etc. He is/has been contributing as technical editor for 6 research journals published from various provinces of Pakistan and is consultant editor for Pakistan Journal of Parasitology, University of Karachi. At Sindh Agriculture University Tandojam, he produced a significant number of MSc, MPhil including 3 Ph.Ds. Dr. Arijo has four published books and 3 practical manuals to his credit and is author and co-author of many publications that have appeared in international and national journals.

Dr. Arijo's research areas have been Schistosomiasis and Tick-borne Diseases. He has completed research projects funded by Sindh Agriculture University, Tandojam and University Grants Commission, Islamabad. He was Principal Investigator of a PAK-US Science & Technology Research Project and has established a State-of-Art Molecular Parasitology Laboratory at Sindh

Agriculture University Tandojam. During this project, he developed collaboration with the University of Rhode Island USA for developing an anti-tick vaccine. Recently, he has been working as Co-PI on a research project entitled "Induced pluripotent stem cells for cardiac cell replacement therapy" with a capital cost of € 37200/- with Professor Kurt of University of Cologne, Germany. In 2015, with the collaboration of Dr. Fateh Uddin Khand, Dr. Arijo was awarded another research project "Studies on Dissolution and Prevention Effects of Hajrul Yahood, Sang Sarmahi, Phyllanthus Niruri on Calcium-Containing Kidney

Stones in Rats" with a total cost of Rs: 7,798,375 funded by HEC Islamabad. To his credit he also got the approval of development scheme for SAU Tandojam with a capital cost of Rs.874 million, as Director Planning & Development of the university during 2014-2017.

Dr. Arijo is on the review committee(s) of the Georgia National Science Foundation Georgia, Pakistan Science Foundation Islamabad, Pakistan Agriculture Research Council, Islamabad-Pakistan. Dr. Arijo has also edited a monograph on "Invertebrate Zoology "by Higher Education Commission, Islamabad. In 2007, his name was included in the Directory of The Productive Scientists of Pakistan. In 2009, he received Presidential Award "Izaz-i-Fazeelat" for his Academic achievements.

In 2015, he got Zoologist of the Year Award given by the Zoological Society of Pakistan. In 2016 he received HEC's Best University Teacher Award with a cash prize of Rs.100,000.

Prof. Dr. Muzaffer Ahmad Lifetime Achievement Award 2023

Prof. Dr. Syed Muhammad Akram Shah,
Department of Zoology,
Qurtuba University of Science &
Information Technology,
Former Professor and Chairman,
Department of Zoology,
University of Peshawar

Prof. Dr. Syed Akram Shah born on 7 February 1960, obtained M.Sc. degree in Zoology with specialization in Parasitology/Zoology from University of Peshawar in 1986 and Ph.D. in Parasitology from Antileishmanial Drug Delivery Lab of Department of Parasitology, School of Life, Basic Medical and Health Sciences, King's College London, University of London in 1993. Prof. Shah spent four years (2002-2006) as post-doctoral researcher in the School of Life, Basic Medical and Health Sciences, King's College London, University of London working on molecular mechanism of diabetic nephropathy. He spent two months in 2009 in School of Biomedical and Health Sciences Hodgkin Building, London Bridge, London working on HMCL apoptosis under hyperglycemic and hyperlipidemic conditions. Prof. Shah collaborated with Prof. Colin Sutherland and Prof. Simon Croft of London School of Hygiene & Tropical Medicine from 2011-2015 Under HEC-BC International Strategic Partnerships in Research and Education programme on a research project "Cutaneous Leishmaniasis in Khyber Pakhtunkhwa (Pakistan): Assessment of antileishmanial and antimicrotubule properties of synthetic buparvaquone in vitro". Another research project entitled "Investigation on the role of arginase-mediated L-arginine on Metabolism in the exacerbation of cutaneous leishmaniasis in pregnancy" worth 7.1 million was later awarded to him by HEC which focused on understanding molecular mechanisms involved in antileishmanial drugs related apoptosis and survival of Leishmania tropica causing Cutaneous Leishmaniasis.

Prof. Dr. Muzaffer Ahmad Lifetime Achievement Award 2023

Dr. Muhammad Afzal Ghauri,
House No.78, Street No.8,
Sector J, DHA 2, Islamabad

Dr. Muhammad Afzal Ghauri obtained his M.Sc. degree in Zoology from University of the Punjab, Lahore in 1986 and Ph.D. degree in Biotechnology/Microbiology from University of Wales, UK in 1991. Dr. Ghauri joined National Institute for Biotechnology and Genetic Engineering, Faisalabad, as Senior Scientist in February 1993. In December 2001 he was promoted as Principal Scientist and in 2013 as Deputy Chief Scientist. He remained Head of Division of Industrial Biotechnology from 2001 to 2021. His R&D interests have been in the areas of Biotechnology of Extremophiles, Bacterial Biodiversity and Systematics, Gene Mining, Bioresource Development for Industry, Bioprocessing of Minerals and Fossil Fuels, Biotechnology of Prebiotics and Antimicrobials and related areas. Being Head of Industrial Biotechnology Division, he was intimately involved in bioprocessing of ores and fossil fuels, biotechnology of prebiotics and antimicrobials, industrial enzymes, fermentation technology, bioenergy and nanobiotechnology. He also extended his expertise in designing bioprocesses and consultancy services to other professional and commercial organizations/industries. During various scientific expeditions, he isolated more than 100 microbial species/strains from different extreme habitats of Pakistan including Khewra Salt Mines, D. G. Khan Uranium Mines, leather tanneries and other rhizospheres. These studies led to the isolation of two Acidithiobacillus ferrooxidans cultures from local environments and now part of National Collection of Industrial and Marine Bacteria (NCIMB), United Kingdom. His post-doctoral period in Leicester University, UK enabled him to develop the area of gene mining by integron capture and to deposit more than 60 genes of varied nature to GenBank, USA as public resource domain. Moreover, he had been involved in optimizing various process parameters for coal biodesulfurization on laboratory and pilot scales and has demonstrated the said process at commercial level (300 tonnes) in collaboration with Askari Cement, Nizampur, Nowshera.

Dr. Ghauri has published his work in the journals of national and international repute. Based on these publications, he has acquired Journal Citation Report (JCR) Impact Factor of 255.582 and Citations ~3000. He has won several research proposals from national and international funding agencies including MoST and HEC (Govt of Pakistan), Third World Academy of Sciences (TWAS Italy) etc. Dr. Ghauri has played an active role in national/international training workshops at NIBGE. Dr. Ghauri has supervised 11 PhD and 17 MPhil students during his career.

Besides scientific excellence, Dr. Ghauri has also published six books on poetry and was awarded SHARIF KUNJAHI Literature Award for the year 2017.

Certification of Appreciation
In Recognition of this Contribution
Towards Promotion of the Subject
of Fisheries and Aquaculture
2023
Dr. Zafar Iqbal
Institute of Zoology
University of the Punjab, Lahore

Dr. Zafar Iqbal graduated from F.C. College Lahore in 1981. He obtained M.Sc. Zoology degree from Government College, Lahore in 1984 and Ph.D degree in the field of Aquaculture / Freshwater Fish Parasitology from the Institute of Aquaculture, University of Stirling, Scotland, UK in 1998. Dr. Zafar Iqbal joined Punjab Fisheries Department as Assistant Director in 1986. He worked for carp hatchery management, extension, conservation and development of fisheries in the Province of Punjab. He later became in-charge of Fish Pathology Lab. at Fisheries Research and Training Institute, Lahore, where he worked extensively on 'Fish Disease diagnostics', teaching and research in Fish Health Management.

In 2003, Dr. Zafar Iqbal, Joined University of the Punjab as Assistant Professor of Zoology and later he was selected as Associate Professor in 2005. He established state of the art 'Fish Disease and Health Management Lab'. He supervised research work of 47 research students including 4 PhD and 43 MS/MPhil students in the area of Fisheries / Fish Diseases. He has published 64 research papers in International and National peer reviewed Journals. He has attended 43 International and National Conferences abroad and in Pakistan. He has written a book chapter on 'Fish Diseases and Health Management' in USDA funded book project, Feeding Pakistan/ Aquaculture Hand Book.

Dr. Zafar Iqbal has been active member of many professional bodies: British Society of Parasitology, Asian Fisheries Society, Biological Society of Pakistan, Fisheries Society of Pakistan, Life fellow Zoological Society of Pakistan. Recently, he received a certificate of appreciation and a shield in recognition of his services in the Field of Fish Disease and Health Management in IFAC- 2023 in Lahore.

Zoologist of the Year Award 2023
Prof. Dr. Zulfiqar Ali
Institute of Zoology
University of the Punjab, Lahore

Dr. Zulfiqar Ali obtained his MSc degree in Zoology from University of the Punjab in 1991 and Ph.D degree from University of the Punjab in 2005. He has written more than 170 articles and books related to wildlife animals with 152 Impact Factor publications and 1689 citations. He has supervised research work of 28 Ph.D and 63 M.Phil students besides 64 MSc/BS graduates. He is founder of the New Zoological Museum in Punjab University, Wildlife Department in UVAS, and provided services to many national and international organizations with his expertise in wildlife management. His work and expertise lie in the area of wildlife conservation that includes: wildlife-surveys, habitat assessment, wetlands management, community mobilization, and sustainable natural resource planning backed by the requisite academic qualification. He has developed and implemented plans to reduce the negative impacts of human activities on biodiversity. His additional expertise as environmental health specialist also with many international research articles and a text book of air pollution monitoring.

Prof. Ali is Member of Aerosol Society UK, British Ecology Society, UK, Linnaean Society of London, UK, Society of Wetlands Scientist, USA, British Natural History Museum, UK, Society for Ecological Restoration International, USA, and WWF-Pakistan. Prof. Ali is also Fellow of Zoological Society of London, UK and Life Fellow of Zoological Society of Pakistan.

Prof. Ali was awarded WWF-International Prince Bernhard Award for Nature Conservation in 2002. He has attended 23 international and 31nationals conferences, seminars and workshops with presentations.

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*Other applicants of this award were. none

Prof. Dr. A.R. Shakoori Gold Medal 2023

Dr. Kanwal Rehman
Chairperson
Department of Pharmacy
The Women University, Multan

Dr. Kanwal Rahman did her PhD in Pharmacology from Zhejiang University, China in 2015. She has published 150 research articles in peer-reviewed journals with cumulative IF 550 and about 7000+ citations with h-index 42. Besides this, she has also published 6 books with international publishers (Springer-Nature and Elsevier) along with 55 book chapters that have been published in various books. She has also supervised 2 PhD and 25 MPhil students. She has also completed 04 HEC funded research grants. She has also won an international research project from the Deanship of scientific research, Jouf University, Kingdom of Saudi Arabia. She is also an awardee of Research Productivity Award from Pakistan Council of Science and Technology and has been recognized among the top 2% of Productive scientists of Pakistan by PCST. Currently, she is also serving as the Associate Editor of 4 peer-reviewed journals. She has also organized several scientific seminars, symposiums and workshops.

Her current research work includes evaluating natural biogenic compounds derived from food and commercially available plants as treatment strategies against different risk factors of metabolic disorders including pancreatitis, cardiometabolic disorders and hormonal imbalance. The main research interest focuses on epidemiology, pathogenesis of metabolic disorders and identification of their risk factors including environmental pollutants and endocrine disruptors investigating their association with gene SNP.

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*Other applicants of this award were. Dr. Saadullah Khan, Dr. Muhammad Khan, Dr. Asif Nadeem, Dr. Wali Khan, Dr. Muzafar Shah

Prof. Dr. A.R. Shakoori Gold Medal 2023

Dr. Muhammad Zaffar Hashmi,
Department of Chemistry
COMSATS University, Islamabad

Dr. Muhammad Zaffar Hashmi joined University of Lahore as an Associate Professor in 2023. Dr. Hashmi received Ph.D. in Environmental Biology from Zhejiang University, China in 2015. He has served as Distinguished Visiting Professor at NIDA Thailand and Professor at Northeastern University China.

Dr. Hashmi is Fellow of Society of Environmental Geochemistry and Health (international), and lifetime member of Pakistan Nuclear Society and Chemical Society of Pakistan. He is member of Pakistan Academy of Sciences and Society of Toxicology.

Dr. Hashmi has research interest to develop treatment technologies to control environmental and industrial pollution; to understand toxicology mechanisms of e-waste and to develop novel methods for monitoring of environmental pollution.

Dr. Hashmi is Editor-in-Chief of two book series: Advances in Pollution Research by Elsevier and Emerging Contaminants and Associated Treatment Technologies by Springer. Dr. Hashmi has published 13 books with Springer and 2 books with Elsevier, 27 book chapters and 124 SCI papers having a cumulative impact factor of 600+ and h-index=30 (Web of Science), h-index=34 (Google-scholar). Dr. Hashmi has won Mustafa Science and Technology Foundation Iran award KANs Prize in Environment and Water (OIC Countries) is prominent and Gold Medal from Pakistan Academy of Sciences.

Dr. Hashmi organized international workshop through Society of Environmental Geochemistry and Health platform. Delivered several talks as keynote speaker and invited speaker in international conferences. Dr. Hashmi is Associate Editor of Environmental Geochemistry and Health, Arabian Journal of Geosciences and Chemosphere etc. He was awarded research grants (15 M PKR) as Principal Investigator from TWAS, HEC, PSF and NSF China. Dr. Hashmi has worked as a consultant for Indus River Disaster Management Project of WWF-Pakistan in 2011.

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*Other applicants of this award were. Dr. Saadullah Khan, Dr. Muhammad Khan, Dr. Asif Nadeem, Dr. Wali Khan, Dr. Muzafar Shah

**Prof. Dr. Mirza Azhar Beg
Gold Medal
2023**

Dr. Hafiz Abdullah Shakir,
Institute of Zoology,
University of the Punjab, Lahore

Dr. Hafiz Abdullah Shakir is serving as Associate Professor in Institute of Zoology, University of the Punjab, Lahore, Pakistan. Dr. Shakir received his B.S. (Hons.) and M.Sc. (Hons.) degrees from the University of the Punjab, Lahore. In 2008, he won an Indigenous Scholarship from the Higher Education Commission for Ph.D. studies in University of the Punjab, Lahore and then awarded IRSIP Scholarship from HEC, Pakistan for the execution of Ph.D. research work in Newcastle University, UK. During his Ph.D research, Dr. Shakir worked on water pollution and fish health.

Dr. Shakir's research areas include Fish Biology, Toxicology and Microbiology. He has published over 110 peer reviewed articles with impact factor >120 in well reputed national and international journals. His research work has been cited more than 950 times in different documents; his h-Index is 17 and i10-index is 29. He has also published 1 book, 5 book chapters, 36 abstracts in international and national scientific conference proceedings. He presented 10 Posters in National/International Conferences/ Symposium/ Seminars.

Dr. Shakir have won 12 research projects including 2 from World Bank sponsored WAPDA projects, 2 from HEC and 1 from PSF, Pakistan. He has supervised research work of 38 MS/M.Phil students, 14 M.Sc./BS students. Four Ph.D. students are working under his supervision. He has delivered over 20 scientific presentations at various national and international meetings/conferences including in UK and Malaysia.

Dr. Shakir is serving as Assistant Editor of Punjab University Journal of Zoology (HEC recognized Journal). He is also In-charge, Punjab University Fish Research Farm.

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*Other applicants of this award were. Dr. Shaista Jalbani, Dr. Zubair Ahmed Laghari,

Prof. Dr. Nasima M. Tirmizi Memorial Gold Medal 2023

Dr. Noor Us Saher,
Centre of Excellence in Marine Biology,
University of Karachi, Karachi

Dr. Noor Us Saher obtained her M.Sc. degree in Applied Zoology in 2001 and Ph.D degree in Zoology with specialization in Marine Zoology from University of Karachi in 2008. Dr. Saher joined the Centre of Excellence in Marine Biology as a faculty member in 2007. The molecular taxonomy of marine species (Decapod crustaceans, Fin fish, Shell fish and Marine snakes) pollution biomarkers, biomonitoring, biochemical variability and characterization of some enzymes and impact of climate change on biodiversity, are the main foci of her research interests. She is the author of more than 400 various nuclear and mitochondrial including, 80 novel genes of various Marine species. She has 80 research papers with an accumulated IF 75, 6 international book chapters and one book as an editor to her credit. She has executed 7 academic and research projects as Principal Investigator and 3 projects as a research officer. She established the Molecular Biology Laboratory in CEMB through HEC NRPU and PSF projects. She has also produced nine Ph.D. scholars as supervisor and four M. Phil. Students.

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* Other applicants of this award were. none

Dr. Abdul Aleem Chaudhary
Gold Medal
2023
Dr. Sangam Khalil,
Department of Forestry,
Rang & Wildlife Management,
Islamia University of Bahawalpur,
Bahawalpur

Dr. Sangam Khalil has done her MSc. in Zoology in 2007, MPhil in 2009 and Ph.D in the field of Wildlife Management from University of Arid Agriculture, Rawalpindi in 2015. Dr. Khalil joined Islamia University Bahawalpur as faculty member in 2016.

She has won Higher Education Commission project grant as well as IDEA WILD USA project grants for conservation of game birds in Pakistan. She has participated in many national and international conferences as presenter and served as invited speaker as well. Published more than 30 research articles in the field of Wildlife Ecology & Conservation.

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*Other applicants of this award were. none

Z.B. Mirza Biodiversity (Faunal) Gold Medal 2023

Dr. Riffat Sultana,
Department of Zoology,
University of Sindh
Jamshoro, Sindh

Dr. Riffat Sultana obtained her Ph.D. degree from University of Sindh, Jamshoro in 2008. She got an experience of post-doctoral research in Chinese Academy of Agricultural Sciences. She joined the Department of Zoology as Assistant Professor in 2010 and then was selected as Associate Professor in 2018. Her expertise's falls in the areas of Biodiversity, Systematics, Biology, Ecology, Chemical & Biological Control and Food preferences of insects in general and Orthoptera in particular. She has described 19 new species and several new records of grasshoppers from sub-continent. Dr. Sultana has 190 published research articles to her credit, both National and International journals of repute. She has accumulated Web of Science IF: 46.07, Google Scholar Citation indices: 705, h-index: 12, i10-index: 24 while in Research Gate citation: Score 896.4, h-index: 14 citation 548, Reads: 41491. She has also written 02 Books & 02 chapters national and internal level 43 Popular Scientific Articles in several dailies Newspapers and Magazines on the subject of pests and pest control. She supervised research work of 17 Ph.Ds. and 50 M.Phils. She was awarded four research projects funded by Higher Education Commission Islamabad and one each by National Geographic Society and Pakistan Science Foundation Islamabad. Recently she signed LOA with FAO for trails of Biopesticides in Pakistan. She is Founder Editor of University of Sindh Journal of Animal Sciences (HEC Recognized) which was launched in 2019.

In 2018 she received 02 Gold Medals - Prof. Dr. Imtiaz Ahmed Gold Medal and Chancellor Gold Medal for outstanding research contributions in the field of Entomology. Beside this, she also received Competitive Research Grant

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*Other applicants of this award were. none

award from Governor of Sindh in 2017, Incentive Award in 2011-2012, certificate of appreciation from Vice Chancellor University of Sindh, Jamshoro in 2017 and Pepsi merit scholarship award for outstanding academic record from Sind Beverage (Pvt). She has attended several International & National scientific conferences, seminars and workshops and presented 350 Research Papers on different aspects of insect's fauna and other burning issues. In view of her expertise and outstanding contributions she was nominated as iBOL Focal Person for Sindh Province-Pakistan by iBOL (International Barcode of Life), University of Guelph, Guelph, Ontario, Canada and was also deputed by Ministry of Food Security, Islamabad as a Sindh Provincial focal person for the management of locust infestation in Sindh, Pakistan.

RECIPIENTS OF
GOLD MEDALS AWARDED BY
THE ZOOLOGICAL SOCIETY OF PAKISTAN

1. **Muzaffer Ahmad Gold Medal 2023**
    The 28th Muzaffer Ahmad Gold Medal 2023 was received by Ms. Sammara Hamid student of University of the Punjab, Lahore for standing first in the recent M.Sc. Zoology examination.

Ms. Sammara Hamid

2. **Afsar Mian Gold Medal 2023**
    The 13th Afsar Mian Gold Medal 2023 was given to Ms. Maria Amjad a student of the Arid Agriculture University, Rawalpindi for standing first in the recent M.Sc. Zoology examination.

Ms. Maria Amjad

3.   Muhammad Afzal Hussain Qadri Memorial Gold Medal 2023
The 24th Muhammad Afzal Hussain Qadri Memorial Gold Medal 2023 was awarded to Muhammad Hashir Nadeem student of Karachi University for standing first in the recent M.Sc. Zoology examination.

Mr. Muhammad Hashir Nadeem

4.   Prof. Dr. S.N.H. Naqvi Gold Medal 2023
The 17th Prof. Dr. S.N.H. Naqvi Gold Medal 2023 was given to Dr. Islam Dad for obtaining Ph.D. degree in Zoology with specialization in the field of Toxicology from University of Karachi.

Dr. Islam Dad

5.   M.A.H. Qadri Memorial Gold Medal 2023
The 21st M.A.H. Qadri Memorial Gold Medal 2023 was given to Dr. Samar Sajjad for obtaining Ph.D. degree in Zoology with specialization in the field of Parasitology from University of Karachi.

Dr. Samar Sajjad

6.   Mujib Memorial Gold Medal 2023
The 28th Mujib Memorial Gold Medal 2023 was given to Khadeeja Ismail a student of Karachi University for standing first in the recent M.Sc. Zoology examination with specialization in Parasitology.

Ms. Khadeeja Ismail

7. Prof. Dr. Muhammad Ali Gold Medal 2023

The 5th Prof. Dr. Muhammad Ali Gold Medal 2023 was awarded to Shumaila Hashim a student of Government College University, Faisalabad for standing first in the recent M.Sc. Zoology examination.

Ms. Shumaila Hashim

8. Prof. Dr. Syed Iftikhar Hussain Jafri Gold Medal 2023

The 5th Prof. Dr. Syed Iftikhar Hussain Jafri Gold Medal 2023 was awarded to Ayesha Paleejo a student of University of Sindh, Jamshoro for standing first in the recent final B.S. Examination of Freshwater Biology & Fisheries.

Ms. Ayesha Paleejo

9.   Ahmed Mohiuddin Memorial Gold Medal 2023
The 22nd Ahmed Mohiuddin Memorial Gold Medal 2023 was awarded to Miss. Manal a student of University of Sindh, Jamshoro for standing first in the recent M.Sc. Zoology examination.

Ms. Manal

10. Prof. Dr. S.S. Akbar Memorial Gold Medal 2023
The 8th Prof. Dr. S.S. Akbar Memorial Gold Medal 2023 was awarded to Mr. Abdul Raheem a student of University of Sindh, Jamshoro for standing first in the recent M.Sc. Zoology examination with specialization in Entomology.

Mr. Abdul Raheem

11. Prof. Dr. Muhammad Saeed Wagon Gold Medal 2023

The 3rd Prof. Dr. Muhammad Saeed Wagon Gold Medal 2023 was awarded to Mr. Noor Muhammad a student of University of Sindh, Jamshoro for standing first in the recent BS-IV Zoology examination.

Mr. Noor Muhammad

12. Prof. Dr. Naeem Tariq Narejo Gold Medal 2023

The 2nd Prof. Dr. Naeem Tariq Narejo Gold Medal 2023 was awarded to Mr. Sarang Samo a student of University of Sindh, Jamshoro for standing first in the recent M.Sc. Fresh Water Biology & Fisheries examination.

Mr. Sarang Samo

SOME GLIMPSES OF ACADEMIC SESSIONS
AND THE CONGRESS PARTICIPANTS

Prof. Dr. Qazi Javed Iqbal escorting the Chief Guest, Prof. Dr. Shahid Munir, Chairman, Punjab Higher Education Committee to the dais

Members of Local Organizing Committee welcoming the guests

Members of local organizing committee welcoming Prof. Dr. Shahid Munir, the Chief Guest

Dr. Abdul Aziz Khan, Dr. A.R. Shakoori, Dr. Shahid Munir and
Dr. Qazi Javed Iqbal are on the dais during inauguration session

Inaugural session: Left to right, Dr. Abdul Aleem Chaudhary
Prof. Dr. A.R. Shakoori, Mr. Abdul Aziz Khan and Mr. Z.B. Mirza

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Chief Guest Prof. Dr. Shahid Munir

Prof. Dr. A.R. Shakoori

**Prof. Dr. Qazi Javed Iqbal welcoming the
Congress delegates**

**Dr. Abdul Aziz Khan addressing the
Inaugural Session**

**Dr. Kanwal Rehman receiving Prof. A.R. Shakoori Gold Medal 2023
from the Chief Guest**

Prof. A.R. Shakoori receiving shield from the Chief Guest

Participants of the Congress

Some participants of the Congress. Prof. Dr. Abdullah G. Arijo, Prof. Dr. A.M. Cheema Dr. Fazle Majid Khan and Prof. Dr. Muhammad Afzal are prominent in the front row

Front row: Lef → Right
Prof. Dr. Rubina Mushtaq (extreme left),
Dr. Nadia Dar is on extreme right

Front row: Lef → Right
Dr. Nadia Dar, Prof. Dr. Farah Rauf Shakoori,
Prof. Dr. Rubina Mushtaq and Prof. Dr. Abida Butt

Some participants of the congress
Dr. & Mrs. Muhammad Khan is on extreme right in the front row

Participants of the congress: Prof. Dr. Zulfiqar Ali,
Dr. Muzaffar Hashmi and Dr. Muhammad Ayub are 3rd, 4th and 5th from the left

Dr. Diyan Li and Dr. Muhammad Akhtar Ali

Lef → Right
Dr. Diyan Li, Dr. Muhammad Akhtar Ali and Prof. Dr. A.R. Shakoori

Prof. Dr. A.R. Shakoori delivering a talk

Dr. Diyan Li delivering a talk

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From left to right: Dr. Diyan Li, Dr. A.M. Cheema and Dr. J.I Qazi

Prof. Dr. Farah Rauf Shakoori with her Ph.D students

Some participants of the Congress
Mr. Abdul Aziz Khan, Mr. Z.B. Mirza, Prof. Dr. Zulfiqar Ali are in the center

Abstracts of Plenary Lectures

PLENARY LECTURE-1

VAGINAL MICROBES AND FEMALE INFERTILITY

Diyan Li

School of Pharmacy, Chengdu University, 2025 Chengluo Avenue, Chengdu, Sichuan, China
Corresponding Author: lidiyan860714@163.com

The vaginal microbiota plays an essential role in female health and reproduction. A healthy reproductive tract environment helps support successful embryo implantation and maintains pregnancy. The vaginal microbiota composition of females with different infertility diagnoses, such as polycystic ovarian syndrome (PCOS), premature ovarian insufficiency, hydrosalpinx, and endometritis, and the influence of vaginal microbiota on the outcome of in vitro fertilization treatment are still unclear because of the paucity and inconsistency of published data. Here we profile the vaginal microbiota of 1,411 women by sequencing the V3-V4 region of the 16S ribosomal RNA gene. We further clarify the relationship of vaginal bacterial composition with female infertility and 11 clinical and biochemical measurements. Lactobacillus (~78%) dominated the vaginal microbiome and clustered into five types. Type III had higher Gardnerella abundance. Type V women with higher abundances of Streptococcus and Prevotella. Type III and Type V women had poorer pregnancy outcomes. Furthermore, microbiome features associated with infertility were: Prevotella nigrescens with endometriosis, Alloscardovia omnicolens with scarred uterus and Prevotella with adverse pregnancy outcomes. The control and PCOS (polycystic ovarian syndrome) groups had relatively higher IVF outcomes. The vaginal microbiome is associated with fertility, suggesting that vaginal microbes could be used to detect infertility and potentially improve IVF outcomes. The study provides insight into the nature of the vaginal microbiome and its relationship with hormones and IVF outcomes, and the findings suggest that surveying the vaginal microbiota might be useful for the detection of some types of infertility. These profiles will undoubtedly increase our understanding of female vaginal bacteria and their impact on reproductive outcomes.

PLENARY LECTURE-2

ZOONOTIC AND ZOOANTHROPONOTIC TRANSMISSION OF SARS-COV-2

Muhammad Munir

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The emergence of multiple variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) highlights the importance of possible animal-to-human (zoonotic) and human-to-animal (zooanthroponotic) transmission and potential spread within animal species. A range of animal species have been verified for SARS-CoV-2 susceptibility, either in vitro or in vivo. However, the molecular bases of such a broad host spectrum for the SARS-CoV-2 remains elusive. We structurally and genetically analysed the interaction between the spike protein, with a particular focus on receptor binding domains (RBDs), of SARS-CoV-2 and its receptor angiotensin-converting enzyme 2 (ACE2) for all conceivably susceptible groups of animals to gauge the structural bases of the SARS-CoV-2 host spectrum. Data will be presented in the context of existing animal infection-based models to provide a foundation on the possible virus persistence in animals and their implications in the future eradication of COVID-19.

PLENARY LECTURE-3

FUNCTIONAL GENETIC AND COMPUTATIONAL APPROACHES FOR COMPLEX PHENOTYPES AND DRUG SCREENS

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The advent of next generation sequencing technologies and ever decreasing cost of sequencing have made it possible to sequence tremendous amount of genomes. The genome sequencing efforts have already revealed millions of genetics variations of all sorts. The mutant genes found in various disease genomes, contribute to the various complex phenotypes and their contribution and mechanisms remain to be investigated in majority of the cases. The revolutionary CRISPR-Cas based genome editing technologies have facilitated the rapid generation of large number of isogenic models of the various genetic alterations. We have implemented CRISPR-Cas based genome editing to develop various cellular disease models to investigate the contribution to overall phenotype and the mechanism of action of various mutations found in different cancer types. The various omic-approaches were implemented to reveal the differential transcriptomes, proteomes, metabolomes, and chromosomal conformations of the disease models. The mutually exclusive *KRAS* and *BRAF* mutations in colorectal cancer cells were modelled in *BRAF* and *KRAS* driven cancer cells, respectively. These cellular models revealed the differential regulation of carnitine metabolic pathway. The genome sequencing and functional genetics of the variations revealed various potential drug target proteins. The computer aided *in silico* drug screening was successfully used to screen FDA approved drug molecules. The promising drug molecules were successfully validated *in vitro*, by using various biochemical assays. The whole transcriptome analyses were used to study the differential transcriptional regulation after drug treatment to uncover the mechanism of the drug.

PLENARY LECTURE-4

EXTINCTION CRISIS OF ORTHOPTERA: STRATEGIC CONSERVATION PLAN AND FUTURE PROSPECTS

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Orthoptera are a diverse group of insects, which consists of about 29396 (OSF: 2022) hitherto described species. The distribution of the Othoptera is worldwide, occurring in both the new and old worlds. Pakistan stands with 263 well-known species within 49 genera in 8 superfamilies: Tettigonioidea, Schizodactyloidea, Grylloidea, Acridoidea, Pyrgomorphoidea, Tetrigoidea, Eumastacoidea and Tridactyloidea. The majority of species don't cause any significant damage with exception of Desert locust. DL swarms caused 3.4 billion US$ to 10.21 billion US$ over the two agricultural seasons in 2020 and 2021 in Pakistan. In fact, Orthoptera are known to be good indicators for environmental monitoring and assessment, being herbivorous/ carnivorous, they are important for ecosystem functioning, they also provide aesthetic value as the songs of crickets and bush-crickets and sound production is also helpful for species identification, some species are burrower maker, which helps in soil aeration. Unluckily, Orthoptera species are threatened with extinction in globally. This is mainly due to habitat loss as a result of agricultural intensification i-e use of fertilizers, heavy machinery, transformation of grassland, overgrazing, use of pesticides, frequent mowing, land abandonment, increasing wildfire frequencies, and touristic development and urbanization. According to the IUCN Red list, Orthoptera are the most threatened group in invertebrates particularly: *Gryllotalpa* and *Schizodactylus* face a huge risk to survival due to anthropogenic activity globally. Some Indian peoples used *Schizodactylus monstrosus* (Drury, 1773) for traditional beliefs that various parts of their body help to cure several diseases. Lucky, we have 4 representatives amongst 11 in Pakistan. As *Schizodactylus* species have a high level of endemism, with (36.36%) and *Gryllotalpa krimbasi* (11.11%) being endemic to Pakistan. As these species are found nowhere else in the world, Pakistan has a big responsibility to conserve them. This study is an attempt to (i) provide insight into the knowledge gaps regarding the conservation status of Orthoptera, (ii) briefly assess its local impact on the existing fauna, and comparison of diversity loss changed over time (iii) clarify the lack of

awareness in taxonomy, distribution, population trends, ecology and major threats to species (iv) analysis of regional conservation planning through identification of priority geographic areas and habitats. I believe this study will help, making orthoptera a higher conservation priority not only for scientists and decision makers but current knowledge already offers a solid basis for action to increase awareness and conservation of these species on the ground. The data generated from the HEC Project No.14787.

PLENARY LECTURE-5

PRUNUS ARMENIACA A POTENTIAL AGENT FOR DRUG FORMULATION AGAINST CUTANEOUS LEISHMANIASIS: AN EMERGING TROPICAL DISEASE IN PAKISTAN

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Cutaneous Leishmaniasis (CL) is widely scattered manifesting a noticeable epidemiological pattern around the globe, while documented reports are limited in Pakistan, and control strategies are at infancy. The present study aimed to investigate the molecular-epidemiology, associated risk factors, and experimental strategy of the leaves extract fractionated of *Prunus armeniaca* for anti-leishmanial activity. The strategies involved (1) a questionnaire for the collection of information and clinical diagnosis (microscopy and semi-nested PCR) of CL symptomatic patients in District Dir, and Kashmir, Khyber Pakhtunkhwa, Pakistan, (2) previous records of CL in Pakistan was studied, and spatial analysis was performed on elevation and agro-ecological maps using Arc-GIS v10.3.1, and (3) fraction characterization by FTIR, UV–Vis, and GC-MS analysis. Active lesions were found predominant than scars, and infected age groups were found significantly different. Majority of the lesions were dry, single, and frequently infecting facial region. Avoiding bed nets, living in mud houses and animal shelters were highly associated with CL infection. Microscopically, less cases were positive, while the PCR assay revealed *Leishmania tropica* in all cases. Throughout Pakistan, prevalence of CL ranges between 5-95% and the main causative agent was *Leishmania tropica*, followed by *L. major* while fewer cases were infected with *L. infantum* and *L. donovani*. Geographically, ZCL due to *L. major*, were more prevalent in the lowland regions of Pakistan, while ACL due to L. tropica was predominant in the highland regions. Dry mountains and plateaus of northwestern and southwestern regions are spatially at high risk. All derived fractions exhibited toxicity in the safety range IC50 > 100 μg/ml. The fraction (F7) showed significantly the highest antipromastigotes activity with IC5011.48 ± 0.82 μg/ml and antiamastigotes activity with IC50 21.03 ± 0.98 μg/ml compared with control i.e. 11.60 ± 0.70 and 22.03 ± 1.02 μg/ml respectively. The GC–MS chromatogram exhibited the presence of

nine essential compounds in which the 1, 2-benzenedicarboxylic acid, diisooctyl ester bound well to the PTR1 receptor. Measures should be taken to reduce CL infection by eliminating the associated risk factors, promoting PCR-based diagnosis and basic medical facilities, and the in vivo studies are required for further unravelling of therapeutic drugs.

PLENARY LECTURE-6

SCIENTIFICALLY STRENGTHENING THE DWINDLING TRADITIONAL MEDICINAL PRACTICES AND LOCAL WISDOMS

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Before the advent of rapid transportation and communication systems which have now been culminated to the Global village world; indigenous/regional knowledge was well in practice and had evolved through human experiences and observations. Such practices formed the basis of established usage of natural resources for sustaining human health as well as to treat diseases. However, the invasion of our region by British empire certainly brought scientifically and experimentally supported solutions to the health sectors, specifically. During the mid of last century provisions of the antibiotics really challenged almost all the available local remedies. Whereas, it was required to scientifically verify and screen the locally established medicinal practices; as they had long been experienced by human wisdom. Now due to rapid information storm through internet facilities our traditional medicinal practices and wisdoms are dwindling from our memories. It is high time to record such folk wisdoms and colour them with scientific progress and verification. Through such efforts, scientifically proven practices will be having a support of already tested human trials. In this paper some examples of traditionally practiced / well known health sustaining and treatment practices will be explained in the light of modern scientific developments which will provide hypotheses for future research to be done in this regard.

PLENARY LECTURE-7

ROLE OF SLIT2-ROBO1 AND RhoA cAMP SIGNALING IN CANCER CELL TYPE DEPENDENT METASTATIC PHENOTYPE

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Epithelial to mesenchymal transition (EMT) is considered as a prerequisite for metastatic dissemination of cancer. The propagating cancer cells attain mesenchymal traits in a multistep process mediating several structural and functional alterations. This process of proliferation in particular is characterized by loss of epithelial markers including E-cadherin and gain of mesenchymal markers expression (e.g., N-cadherin, vimentin, etc). The regulatory events induce amendments in cell-cell and cell-matrix adhesion followed by degradation of extra cellular matrix leading to an increase in migration and augmented invasiveness. Multiple soluble factors, chemokines and growth factors facilitate these transformations. Slit-Robo signaling was reported to play key roles in regulation of these events by mediating expression of Rho GTPases as well as EMT marker genes. But the role of Slit in particular is paradoxical in cancer. As in some cancer types Slit 2 acts as anti-tumorigenic while in others it is contributing towards the metastatic phenotype. The present study was aimed at deciphering the ambivalent metastatic attribute of Slit2 by analyzing the role of different modulators on Slit-Robo mediated carcinogenesis, in particular emphasizing the role of cAMP/RhoA signal transduction. In our study IBMX (3-Isobutyl-1-methylxanthine) and two growth factors (TGF-β and FGF2) were used and their potential effect was evaluated on the carcinogenic capacity of Slit2. Upon results interpretation it was unraveled that the cell migration and proliferation was increased in colon cancer cells after Slit2 administration and decreased in cervical cancer cells. But Slit2 is definitely playing important role in cancer regulation as its exposure affected cell morphology as well as cell progression in both cancer types. The oncogenic phenotype was further increased in presence of TGF-β and FGF2. IBMX however contributed to decrease Slit mediated migratory elevation. The

anti-tumor idiosyncrasy of Slit2 though is cancer type specific and various neighboring regulatory elements are involved in mediating Slit-Robo signaling. Our data however concluded that Slit2 is definitely a potential biomarker in cancer diagnosis and therapeutic targeting and along with the regulatory molecules it can be employed in strategic prognostic procedures.

PLENARY LECTURE-8

AIR POLLUTION: EFFECT OF TOXIC ELEMENTS ON HEALTH IN PAKISTAN

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Pakistan's contribution to climate change in terms of greenhouse effect is less than 1%, however this country has become the 5th most vulnerable country in the world to changes in climate. The unprecedented unseasonal heavy rains and floods during 2022 have confirmed our vulnerability to climate change. Environmental pollution, especially the air pollution has been the cause of millions of premature deaths in this region. A recent study by the University of Chicago has shown a decrease of 3.8 years in life expectancy of Pakistanis due to air pollution alone. Smog resulting from emissions of vehicles, industry, brick kilns and crop stubble burning has been the major cause of respiratory diseases, allergies and eye diseases during the winter season. The most lethal components of smog include fine particulate matter PM2.5 and PM10 and "soot"- an impure carbon. In addition to fine particulate matter, toxic heavy metals emitted from vehicles, industry and mining activities further add to the environmental pollution in the country. These include, arsenic, cadmium, mercury, lead, iron, chromium, copper and nickel. Intake of some of these heavy metals cause GI problems, cardiovascular diseases, leukemia and cancer. These toxic metals are not only affecting the health of people of Pakistan, but are also damaging the crops and harming the aquatic life. The only way to mitigate the ill-effects of these toxic elements and air pollution is to make Pakistan "Clean and Green". This would require a multi-pronged approach in which all stakeholder would be expected to play a proactive and significant role to protect the future generations in Pakistan from devastations of climate change.

PLENARY LECTURE-9

ECOLOGICAL SERVICES PROVIDED BY SPIDERS IN AGROECOSYSTEMS OF PAKISTAN

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Spiders are a diversified, abundant and ubiquitous group of invertebrates in terrestrial and aquatic ecosystems. They have a wide insect host range and can kill large numbers of insect pests in agroecosystems. Their habitat preferences, prey searching ability, and polyphagus nature make them good biological suppressors of insect pests. Our studies provide strong evidence that spiders are effective in natural pest control and improve crop performance. However, the efficacy of spiders differed among crops. Spider pest suppression efficacy increased with taxonomic diversity and density of active hunters in the fields. The effects of spiders cascaded down and improved crop performance. However, their biocontrol functioning is influenced by characteristics of crops, climatic conditions and different agricultural practices. Our agroecosystem is continuously loaded with different xenobiotics released by different anthropogenic activities. Biosensors or organisms that respond to these xenobiotics in some measurable ways are useful tools to study the effects of these chemical pollutants at different levels of biological organization. Spiders are excellent bioindicators of pollutants in ecosystems because they are sensitive to natural and anthropogenic disturbances. Studies of my lab propose that spider species provide trustworthy evaluation of the habitat status in relevance to the heterogeneity and disturbance gradient. Spider assemblages are well appropriate to differentiate habitat quality, since many spiders depend on a distinct complex of environmental and habitat factors with regard to species-specific ecological demands and tolerance. In Pakistan, studies related with the functioning of spiders in agroecosystems are very few. Further studies are required to use the potential of spiders as biomonitoring and biocontrol agents in agroecosystems.

PLENARY LECTURE-10

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PLENARY LECTURE-11

RESEARCH PROGRESS OF LIVER AQUAPORIN

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Aquaporins (AQPs) are cell membrane integral proteins that mediate efficient transmembrane water transport. There are 13 members of the mammalian AQP family (AQP0~AQP12), which are selectively expressed in the form of homologous tetramers on the plasma membrane and intracellular membrane structures of certain types of cells in various organs, where they mediate efficient transmembrane water moving pathway driven by osmotic pressure or facilitate sensitive volume regulation. In addition to the efficient transport of water molecules, some AQP family members can also permeate some neutral small molecule solutes such as glycerol and urea and designated as aquaglyceroporins. In recent years, it has been found that many AQP family members can efficiently transport H_2O_2 and mediate the flow of H_2O_2 inside and outside cells or between organelles and cytoplasm. These AQPs are now called peroxiporins.

The expression and function of AQPs in the liver have attracted increasing interest in the field. Some studies suggested that AQP family members may have a key role in the regulatory mechanisms of hepatic bile secretion, glycose/lipid metabolism and oxidative stress. Our laboratory has conducted a series of studies on the expression and function of liver aquaporins in recent years. Our studies revealed important roles of AQPs AQP8 and AQP9 in the mechanisms of bile secretion, gallstone formation, liver regeneration and alchoholic liver injury. In addition, we found AQP7 expression in hepatic stallete cells where it regulates fat metabolism and vitamin A release and is involved in cholestatic liver injury and fibrosis. These findings demonstrated that AQPs are involved in broad physiological and pathological mechanisms of liver by facilitating cellular water, glycerol and H_2O_2 transport.

PLENARY LECTURE-12

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