ISSN Print: 2518-4261
ISSN Online: 2518-427X
Vol. 59(2), June 2022

PROCEEDINGS
OF THE PAKISTAN ACADEMY OF SCIENCES:
B. Life and Environmental Sciences

PAKISTAN ACADEMY OF SCIENCES
ISLAMABAD, PAKISTAN

Proceedings of the Pakistan Academy of Sciences: Part B Life and Environmental Sciences

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Published by **Pakistan Academy of Sciences**, 3 Constitution Avenue, G-5/2, Islamabad, Pakistan
Tel: 92-51-9207140; 92-51-920 6770; **Websites:** www.paspk.org/proceedings/; www.ppaspk.org

Printed at **Graphics Point.,** Office 3-A, Wasal Plaza, Fazal-e-Haq Road, Blue Area, Islamabad
Ph: 051-2806257; E-mail: graphicspoint16@gmail.com

PROCEEDINGS OF THE
PAKISTAN ACADEMY OF SCIENCES: PART B
Life and Environmental Sciences

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Proceedings of the Pakistan Academy of Sciences: Part B
Life and Environmental Sciences 59(2): 1-17 (2022)
Copyright © Pakistan Academy of Sciences
ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(59-2)683

Pakistan Academy of Sciences

Bibliometric Analysis of Proceedings of the Paksitan Academy of Sciences: Part B from 2016 to 2021

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Proceedings of the Pakistan Academy of Sciences: Part B (Life and Environmental Sciences) is the official flagship journal of the Pakistan Academy of Sciences. It publishes in the fields of agricultural, biological, environmental and health sciences. Scopus database is directly covering it since 2016 and till 19th April 2022, it has published 210 research documents majorly comprising of articles (n=141), book chapters (n=36), conference papers (n=26), reviews (n=6) and one (n=1) note. It also received 232 total citations. We extracted the publication data from Scopus in BibTeX format and analyzed it on R-Studio. In all publications, 313 authors from 278 institutes or universities from 14 Asian, 6 European, 2 Middle East, 1 Oceanic (Australia), 2 North American, 1 South American (Brazil) and 3 African countries have contributed. The country co-authorship network (constructed on Vosviewer) is presented in Supplemnetaty data (Figure 1). The lists of all authors (with total publications (TP), total citations (TC), publications years, h-index, g-index and m-index), all universities (with TP) and countries (with TP) are provided in supplementary data (Table 1, 2 & 3). It has achieved considerable CiteScore (0.6), SJR (0.143) and SNIP (0.347) calculated on 05th May 2022 by Scopus for the year 2021. The success could be attributed to the editorial board (which has experts from Pakistan, Australia, Canada, China, USA, Turkey, Oman, Malaysia, and Indonesia), reviewers, authors, and editorial management. The number of publications, citations and its foothold in different countries confirm that the journal's reputation is significantly improving.

Keywords: PPAS B, Scopus, and Publications

Received: May 2022; Accepted: June 2022
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Supplementary Data

Table 1. List of all authors with the total number of publications (NP), total citations (TC), h-index, g-index, and m-index

Table 2. List of all universities with a total number of publications (NoP).

Table 3. List of all countries with a total number of publications (NoP).

Figure 1. Country co-authorship network (constructed on Vosviewer)

Table 1. List of all authors with the total number of publications (NP), total citations (TC), h-index, g-index, and m-index

S. No.	Name of Author	NP	TC	h_index	g_index	m_index	PY_start
1.	ABBAS A	1	1	1	1	0.143	2016
2.	ABBAS K	2	7	2	2	0.4	2018
3.	ABBAS M	1	1	1	1	0.2	2018
4.	ABBAS Q	1	3	1	1	0.25	2019
5.	ABBAS SQ	1	1	1	1	0.143	2016
6.	ABBASI FM	1	2	1	1	0.167	2017
7.	ABBASI MK	1	1	1	1	0.25	2019
8.	ABDULLAH S	2	7	2	2	0.4	2018
9.	ABID A	1	3	1	1	0.25	2019
10.	ABRO SH	1	2	1	1	0.25	2019
11.	ABRO ZUA	1	1	1	1	0.167	2017
12.	ABUZAR MK	1	8	1	1	0.167	2017
13.	ADHIM ATF	1	3	1	1	0.167	2017
14.	ADINURANI PG	1	13	1	1	0.167	2017
15.	AFAQ M	1	2	1	1	0.143	2016
16.	AFRIDI S	1	2	1	1	0.25	2019
17.	AHMAD B	1	8	1	1	0.167	2017
18.	AHMAD F	2	3	1	1	0.143	2016
19.	AHMAD G	1	2	1	1	0.143	2016
20.	AHMAD KS	1	1	1	1	0.2	2018
21.	AHMAD M	1	2	1	1	0.25	2019
22.	AHMAD MM	2	9	1	2	0.143	2016
23.	AHMAD W	1	2	1	1	0.25	2019
24.	AHMED E	1	1	1	1	0.143	2016
25.	AHMED I	3	41	3	3	0.429	2016
26.	AHMED M	1	5	1	1	0.167	2017
27.	AHMED MM	1	1	1	1	0.2	2018
28.	AHMED S	1	4	1	1	0.167	2017
29.	AHMED SN	1	2	1	1	0.25	2019
30.	AHSAN A	1	2	1	1	0.25	2019
31.	AJAIB M	1	4	1	1	0.25	2019
32.	AKBAR M	1	1	1	1	0.143	2016

S. No.	Name of Author	NP	TC	h_index	g_index	m_index	PY_start
33.	AKBAR W	1	1	1	1	0.167	2017
34.	AKHTAR ALI SHAH S	2	8	1	2	0.143	2016
35.	AKHTAR S	1	4	1	1	0.143	2016
36.	AKRAM F	1	2	1	1	0.25	2019
37.	ALI A	1	1	1	1	0.25	2019
38.	ALI F	1	1	1	1	0.2	2018
39.	ALI K	1	3	1	1	0.25	2019
40.	ALI M	2	3	1	1	0.143	2016
41.	ALI S	1	2	1	1	0.25	2019
42.	ANANDITO RBK	1	5	1	1	0.167	2017
43.	ANDINI A	1	1	1	1	0.5	2021
44.	ANGGORO S	1	3	1	1	0.2	2018
45.	ANJUM F	1	1	1	1	0.25	2019
46.	ANNE O	1	4	1	1	0.2	2018
47.	ANUM K	1	2	1	1	0.25	2019
48.	ANWAR E	1	15	1	1	0.167	2017
49.	ANWAR MM	1	5	1	1	0.167	2017
50.	ANWAR T	1	1	1	1	0.25	2019
51.	AROOJ A	1	7	1	1	0.167	2017
52.	ARSHAD F	1	1	1	1	0.25	2019
53.	ARSHAD M	1	1	1	1	0.167	2017
54.	ARSHAD R	1	2	1	1	0.2	2018
55.	ASGHAR M	1	2	1	1	0.25	2019
56.	ASHFAQ S	1	2	1	1	0.25	2019
57.	ASHRAF A	2	9	1	2	0.143	2016
58.	ASHRAF H	1	5	1	1	0.167	2017
59.	ASLAM A	1	3	1	1	0.143	2016
60.	ATTA-UR-RAHMAN AR	2	8	1	2	0.143	2016
61.	ATTA-UR-RAHMAN AUR	3	13	3	3	0.429	2016
62.	AYAZ M	1	2	1	1	0.25	2019
63.	AZAM S	1	4	1	1	0.25	2019
64.	AZIZ I	1	3	1	1	0.25	2019
65.	AZIZAH LH	1	1	1	1	0.2	2018
66.	BALAL RM	1	1	1	1	0.167	2017
67.	BALOCH N	1	1	1	1	0.167	2017
68.	BASHIR S	1	2	1	1	0.25	2019
69.	BATOOL Y	1	5	1	1	0.2	2018
70.	BHOWMIK KR	1	1	1	1	0.143	2016
71.	BIDDINIKA MK	1	9	1	1	0.167	2017
72.	BOKHARI SAI	1	15	1	1	0.167	2017
73.	BUTT MS	1	2	1	1	0.25	2019
74.	CHANDIO A	1	2	1	1	0.25	2019
75.	CHANNA IA	1	2	1	1	0.25	2019

S. No.	Name of Author	NP	TC	h_index	g_index	m_index	PY_start
76.	COLLINS AE	1	5	1	1	0.167	2017
77.	DARBAN DA	1	1	1	1	0.143	2016
78.	DIN M	1	2	1	1	0.25	2019
79.	FAISAL R	1	3	1	1	0.25	2019
80.	FARMAN M	1	2	1	1	0.167	2017
81.	FAROOQ S	1	4	1	1	0.25	2019
82.	FARZANA F	1	1	1	1	0.143	2016
83.	FATIMA H	1	1	1	1	0.2	2018
84.	FATIMA R	1	3	1	1	0.143	2016
85.	FATMAWATI S	1	2	1	1	0.25	2019
86.	GHANI A	1	1	1	1	0.2	2018
87.	GIYARSIH SR	1	1	1	1	0.25	2019
88.	HABIB M	1	5	1	1	0.143	2016
89.	HABIB U	1	5	1	1	0.143	2016
90.	HAFEEZ S	1	1	1	1	0.25	2019
91.	HAI A	1	5	1	1	0.143	2016
92.	HAIDER H	1	1	1	1	0.143	2016
93.	HAIDER SS	1	11	1	1	0.143	2016
94.	HANDAJANI R	1	1	1	1	0.5	2021
95.	HANSEN Y	1	1	1	1	0.333	2020
96.	HARSONO SS	1	13	1	1	0.167	2017
97.	HARTONO S	1	2	1	1	0.2	2018
98.	HASAN B	1	3	1	1	0.143	2016
99.	HASSAN FR	1	4	1	1	0.143	2016
100.	HASSAN GZ	1	4	1	1	0.143	2016
101.	HAYAT MQ	1	15	1	1	0.167	2017
102.	HAYDAR S	2	3	1	1	0.143	2016
103.	HELIYANTO B	1	1	1	1	0.167	2017
104.	HENDRANINGSIH L	1	5	1	1	0.167	2017
105.	HENNISA DL	1	4	1	1	0.2	2018
106.	HIDAYAT T	2	16	1	2	0.167	2017
107.	HIDAYATI L	1	4	1	1	0.2	2018
108.	HUI SIEN K	1	3	1	1	0.2	2018
109.	HUSNI A	1	8	1	1	0.2	2018
110.	HUSSAIN A	7	29	3	5	0.5	2017
111.	HUSSAIN D	1	1	1	1	0.143	2016
112.	HUSSAIN F	1	1	1	1	0.143	2016
113.	HUSSAIN G	3	4	1	1	0.143	2016
114.	HUSSAIN I	1	1	1	1	0.143	2016
115.	HUSSAIN M	2	2	1	1	0.2	2018
116.	HUSSAIN Q	2	9	1	2	0.143	2016
117.	HUSSAIN S	1	3	1	1	0.25	2019
118.	HUSSAIN T	1	4	1	1	0.25	2019

S. No.	Name of Author	NP	TC	h_index	g_index	m_index	PY_start
119.	IBRAHIM MI	1	2	1	1	0.167	2017
120.	ICHSAN MZ	1	1	1	1	0.5	2021
121.	IFTAKHAR T	1	1	1	1	0.143	2016
122.	IKRAM M	1	1	1	1	0.2	2018
123.	ILYAS M	1	2	1	1	0.167	2017
124.	IMRAN M	1	1	1	1	0.2	2018
125.	IMTIAZ A	1	1	1	1	0.2	2018
126.	INAMULLAH I	1	2	1	1	0.167	2017
127.	IQBAL MB	1	1	1	1	0.143	2016
128.	IRAM S	1	1	1	1	0.2	2018
129.	IRFAN M	2	8	1	2	0.167	2017
130.	ISHTIAQ M	1	4	1	1	0.25	2019
131.	ISLAM S	1	1	1	1	0.143	2016
132.	JABBAR S	2	6	2	2	0.333	2017
133.	JACOEB AM	1	1	1	1	0.2	2018
134.	JAFFRI SB	1	1	1	1	0.2	2018
135.	JALEES MI	1	3	1	1	0.143	2016
136.	JAMAL S	1	16	1	1	0.143	2016
137.	JAN SA	2	4	2	2	0.333	2017
138.	JANILA P	1	1	1	1	0.167	2017
139.	KAUSAR T	1	4	1	1	0.167	2017
140.	KAZMI Z	1	4	1	1	0.167	2017
141.	KHALID I	1	3	1	1	0.143	2016
142.	KHALID R	1	1	1	1	0.25	2019
143.	KHALIL AT	2	5	2	2	0.5	2019
144.	KHAN A	1	2	1	1	0.25	2019
145.	KHAN AA	1	1	1	1	0.2	2018
146.	KHAN AB	1	1	1	1	0.143	2016
147.	KHAN AU	1	5	1	1	0.143	2016
148.	KHAN I	1	2	1	1	0.25	2019
149.	KHAN MA	1	7	1	1	0.143	2016
150.	KHAN MUG	1	1	1	1	0.143	2016
151.	KHAN MW	1	1	1	1	0.167	2017
152.	KHAN REA	1	2	1	1	0.167	2017
153.	KHAN SW	1	3	1	1	0.25	2019
154.	KHAN Z	1	3	1	1	0.143	2016
155.	KHATHIAN MA	1	1	1	1	0.25	2019
156.	KHICHI AHK	1	3	1	1	0.167	2017
157.	KHUHRO NH	1	1	1	1	0.167	2017
158.	KHURSHID H	1	2	1	1	0.167	2017
159.	KHUSRO S	1	2	1	1	0.25	2019
160.	KOESNOWIDAGDO S	1	1	1	1	0.5	2021
161.	KUAN LK	1	1	1	1	0.167	2017

S. No.	Name of Author	NP	TC	h_index	g_index	m_index	PY_start
162.	KUMARI N	1	1	1	1	0.333	2020
163.	KUSTIARIYAH K	1	1	1	1	0.2	2018
164.	KUSUMA AF	1	2	1	1	0.2	2018
165.	LAGHARI SK	1	1	1	1	0.143	2016
166.	LATIF M	1	11	1	1	0.143	2016
167.	LI Z	1	20	1	1	0.143	2016
168.	LUTHFIYANA N	1	15	1	1	0.167	2017
169.	MAFTUCHAH M	1	1	1	1	0.167	2017
170.	MAJID A	1	1	1	1	0.2	2018
171.	MALALA AR	1	13	1	1	0.167	2017
172.	MALIK I	1	2	1	1	0.25	2019
173.	MALIK M	1	1	1	1	0.25	2019
174.	MAQBOOL M	1	4	1	1	0.25	2019
175.	MARDIATNO D	1	1	1	1	0.25	2019
176.	MASOOD S	1	2	1	1	0.25	2019
177.	MEHFOOZ B	1	3	1	1	0.167	2017
178.	MEHMOOD W	1	4	1	1	0.167	2017
179.	MEL M	3	16	2	3	0.333	2017
180.	MERAJ MA	1	2	1	1	0.167	2017
181.	MIANA GA	1	2	1	1	0.25	2019
182.	MUNAWAR T	1	1	1	1	0.25	2019
183.	MUSHTAQ S	1	1	1	1	0.143	2016
184.	NADEEM M	2	5	1	2	0.167	2017
185.	NAEEM S	1	1	1	1	0.25	2019
186.	NAKAMURA K	1	3	1	1	0.2	2018
187.	NASEER S	1	3	1	1	0.25	2019
188.	NASRULLAH N	1	2	1	1	0.167	2017
189.	NAUREEN H	1	2	1	1	0.25	2019
190.	NAWAZ S	1	3	1	1	0.143	2016
191.	NAZ H	2	7	2	2	0.4	2018
192.	NELWAN LO	1	13	1	1	0.167	2017
193.	NERGIS Y	1	1	1	1	0.2	2018
194.	NIDA N	1	1	1	1	0.143	2016
195.	NINDITA A	2	15	2	2	0.333	2017
196.	NOERWIJATI K	1	2	1	1	0.167	2017
197.	NOREEN S	1	2	1	1	0.167	2017
198.	NOUREEN S	1	1	1	1	0.2	2018
199.	NUGRAHAENI N	1	1	1	1	0.167	2017
200.	NUGROHO AE	1	8	1	1	0.2	2018
201.	NURHAYATI T	1	1	1	1	0.2	2018
202.	NURILMALA M	1	15	1	1	0.167	2017
203.	NURJANAH N	1	15	1	1	0.167	2017
204.	OMAR MS	1	3	1	1	0.2	2018

S. No.	Name of Author	NP	TC	h_index	g_index	m_index	PY_start
205.	OVAIS M	1	2	1	1	0.25	2019
206.	PAMUNGKAS D	1	5	1	1	0.167	2017
207.	PATOLI AA	1	1	1	1	0.333	2020
208.	PATOLI BB	1	1	1	1	0.333	2020
209.	PERVEEN A	1	1	1	1	0.143	2016
210.	PIGHINELLI L	1	1	1	1	0.5	2021
211.	PRABOWO B	1	9	1	1	0.167	2017
212.	PRAJITNO D	1	2	1	1	0.167	2017
213.	PRATIWI J	1	1	1	1	0.333	2020
214.	PRATIWI T	1	8	1	1	0.2	2018
215.	PRIHANTO AA	1	4	1	1	0.2	2018
216.	PRIHARTINI I	1	3	1	1	0.167	2017
217.	PURBAJANTI E	1	13	1	1	0.167	2017
218.	PURNAMAYATI L	1	5	1	1	0.167	2017
219.	PURNOMO J	1	1	1	1	0.167	2017
220.	PURWANTI F	1	3	1	1	0.2	2018
221.	PURWANTO MGM	1	1	1	1	0.333	2020
222.	PUTRI RF	1	1	1	1	0.25	2019
223.	QADEER A	1	1	1	1	0.25	2019
224.	QAZI IM	2	36	2	2	0.286	2016
225.	QAZI JI	1	7	1	1	0.167	2017
226.	QURESHI H	1	1	1	1	0.25	2019
227.	RABBANI MA	1	2	1	1	0.167	2017
228.	RACHMAWATI D	1	4	1	1	0.2	2018
229.	RAHAYU ID	1	2	1	1	0.25	2019
230.	RAHAYU M	1	1	1	1	0.167	2017
231.	RAHMAN G	2	10	2	2	0.333	2017
232.	RAHSEED R	1	1	1	1	0.25	2019
233.	RAMADHAN SW	1	3	1	1	0.167	2017
234.	RANDHAWA MA	1	2	1	1	0.25	2019
235.	RASHEED A	1	1	1	1	0.25	2019
236.	RASHID MU	1	11	1	1	0.143	2016
237.	RASHID S	1	2	1	1	0.25	2019
238.	RASOOL RM	1	1	1	1	0.25	2019
239.	RASUL G	1	1	1	1	0.143	2016
240.	RATHORE S	1	1	1	1	0.333	2020
241.	RAZA M	1	1	1	1	0.143	2016
242.	RIADI L	1	1	1	1	0.333	2020
243.	RIAZ S	1	1	1	1	0.143	2016
244.	RICO MJI	1	3	1	1	0.167	2017
245.	SAFDAR MN	1	4	1	1	0.167	2017
246.	SAFDAR N	1	4	1	1	0.167	2017
247.	SAHREEN S	1	15	1	1	0.167	2017

S. No.	Name of Author	NP	TC	h_index	g_index	m_index	PY_start
248.	SALEEM MU	1	2	1	1	0.167	2017
249.	SAMIULLAH S	4	16	3	4	0.429	2016
250.	SAMUDRA AG	1	8	1	1	0.2	2018
251.	SASMITO A	1	13	1	1	0.167	2017
252.	SETYOBUDI RH	5	33	4	5	0.667	2017
253.	SHAH JA	1	1	1	1	0.2	2018
254.	SHAH SAA	1	3	1	1	0.143	2016
255.	SHAH SF	1	1	1	1	0.2	2018
256.	SHAH W	1	1	1	1	0.143	2016
257.	SHAHID MA	1	1	1	1	0.167	2017
258.	SHAHZAD N	1	1	1	1	0.143	2016
259.	SHAKIR HA	1	7	1	1	0.167	2017
260.	SHAREEF H	1	1	1	1	0.25	2019
261.	SHARIF M	1	1	1	1	0.2	2018
262.	SHIMIZU K	1	2	1	1	0.25	2019
263.	SHINWARI L	1	3	1	1	0.25	2019
264.	SHINWARI S	1	3	1	1	0.25	2019
265.	SHINWARI W	1	2	1	1	0.25	2019
266.	SHINWARI ZK	3	5	2	2	0.286	2016
267.	SIDDIQUI MA	1	2	1	1	0.25	2019
268.	SIDDIQUI S	1	3	1	1	0.167	2017
269.	SISWANTI S	1	5	1	1	0.167	2017
270.	SODIQ H	1	5	1	1	0.167	2017
271.	SOLHERIA SF	1	2	1	1	0.25	2019
272.	SOMOWIYARJO S	1	2	1	1	0.2	2018
273.	SRI SUMANTYO JT	1	1	1	1	0.25	2019
274.	SRI TUNJUNG WA	1	4	1	1	0.2	2018
275.	SUDARMO H	1	1	1	1	0.167	2017
276.	SULANDARI S	1	2	1	1	0.2	2018
277.	SULTANA S	1	2	1	1	0.25	2019
278.	SUTANTO A	1	2	1	1	0.25	2019
279.	SYAMSIRO M	1	9	1	1	0.167	2017
280.	TABBASUM F	1	7	1	1	0.167	2017
281.	TAHIR MM	1	1	1	1	0.25	2019
282.	TAJ T	1	4	1	1	0.167	2017
283.	TARYONO T	1	2	1	1	0.167	2017
284.	TAUFANI WT	1	3	1	1	0.2	2018
285.	TUFAIL F	1	4	1	1	0.167	2017
286.	UL AIN Q	1	15	1	1	0.167	2017
287.	UL HAQ I	1	2	1	1	0.25	2019
288.	ULLAH A	1	3	1	1	0.25	2019
289.	ULLAH I	1	2	1	1	0.25	2019
290.	ULLAH J	1	20	1	1	0.143	2016

S. No.	Name of Author	NP	TC	h_index	g_index	m_index	PY_start
291.	ULLAH S	3	4	1	1	0.25	2019
292.	UR-REHMAN A	1	1	1	1	0.143	2016
293.	UR REHMAN M	1	2	1	1	0.25	2019
294.	USTADI U	1	8	1	1	0.2	2018
295.	UTARI SA	1	1	1	1	0.2	2018
296.	VINCĒVIČA-GAILE Z	1	5	1	1	0.167	2017
297.	WAHONO SK	1	13	1	1	0.167	2017
298.	WAHYUDI A	1	5	1	1	0.167	2017
299.	WAJIHA H	1	1	1	1	0.143	2016
300.	WASEEM M	1	1	1	1	0.25	2019
301.	WIBIRAMA S	1	1	1	1	0.25	2019
302.	WIDODO W	1	2	1	1	0.25	2019
303.	WINAYA A	1	3	1	1	0.167	2017
304.	YAN M	1	9	1	1	0.167	2017
305.	YAQOOB I	1	4	1	1	0.25	2019
306.	YAR P	1	7	1	1	0.143	2016
307.	YASEEN G	1	2	1	1	0.25	2019
308.	YASMIN A	1	4	1	1	0.167	2017
309.	ZAFAR A	1	1	1	1	0.143	2016
310.	ZAFAR M	2	5	2	2	0.5	2019
311.	ZAFAR U	1	5	1	1	0.167	2017
312.	ZAHEER E	1	1	1	1	0.25	2019
313.	ZAINUDIN A	1	1	1	1	0.167	2017

Table 2. List of all universities with a total number of publications (NoP).

S. No.	Affiliations	NoP
1.	QUAID-I-AZAM UNIVERSITY	16
2.	UNIVERSITY OF PESHAWAR	16
3.	SINDH AGRICULTURE UNIVERSITY	15
4.	KARAKORAM INTERNATIONAL UNIVERSITY	11
5.	PAKISTAN ACADEMY OF SCIENCES	11
6.	UNIVERSITY OF SARGODHA	10
7.	DIPONEGORO UNIVERSITY	9
8.	UNIVERSITY OF THE PUNJAB	9
9.	GOMAL UNIVERSITY	8
10.	THE UNIVERSITY OF AGRICULTURE	8
11.	UNIVERSITAS GADJAH MADA	8
12.	LAHORE COLLEGE FOR WOMEN UNIVERSITY	7
13.	UNIVERSITY OF AGRICULTURE	7
14.	UNIVERSITY OF ENGINEERING AND TECHNOLOGY	7
15.	UNIVERSITY OF MUHAMMADIYAH MALANG	7
16.	SOCIAL SCIENCES RESEARCH INSTITUTE	6
17.	THE UNIVERSITY OF LAHORE	6

S. No.	Affiliations	NoP
18.	UNIVERSITY OF KARACHI	6
19.	GOVERNMENT COLLEGE UNIVERSITY	5
20.	INTERNATIONAL ISLAMIC UNIVERSITY	5
21.	INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA	5
22.	LAHORE GARRISON UNIVERSITY	5
23.	UNIVERSITY OF MANAGEMENT AND TECHNOLOGY	5
24.	UNIVERSITY OF SINDH	5
25.	BOGOR AGRICULTURAL UNIVERSITY	4
26.	FOOD SCIENCE AND PRODUCT DEVELOPMENT INSTITUTE	4
27.	UNIVERSITY OF EDUCATION	4
28.	UNIVERSITY OF SURABAYA	4
29.	AGA KHAN UNIVERSITY	3
30.	AIRLANGGA UNIVERSITY	3
31.	ALTERNATE ENERGY AND WATER RESOURCES INSTITUTE	3
32.	FATIMA JINNAH WOMEN UNIVERSITY	3
33.	GC UNIVERSITY	3
34.	HAZARA UNIVERSITY	3
35.	JINNAH SINDH MEDICAL UNIVERSITY	3
36.	KARAKORAM INTERNATIONAL UNIVERSITY GILGIT-BALTISTAN	3
37.	MA CHUNG RESEARCH CENTER FOR PHOTOSYNTHETIC PIGMENTS	3
38.	MIRPUR UNIVERSITY OF SCIENCE AND TECHNOLOGY (MUST)	3
39.	NATIONAL INSTITUTE OF HEALTH	3
40.	NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY	3
41.	NUCLEAR INSTITUTE OF AGRICULTURE (NIA)	3
42.	QARSHI UNIVERSITY	3
43.	QUAID-E-AZAM UNIVERSITY	3
44.	UNIVERSITY OF AGRICULTURE FAISALABAD	3
45.	UNIVERSITY OF CENTRAL PUNJAB	3
46.	UNIVERSITY OF LAHORE	3
47.	UNIVERSITY OF POONCH	3
48.	UNIVERSITY OF SWABIKP	3
49.	UNIVERSITY OF VETERINARY AND ANIMAL SCIENCES	3
50.	ABDUL WALI KHAN UNIVERSITY	2
51.	ALLAMA IQBAL OPEN UNIVERSITY	2
52.	BACHA KHAN UNIVERSITY	2
53.	BAHAUDDIN ZAKARIYA UNIVERSITY	2
54.	BAHRIA UNIVERSITY	2
55.	BAHRIA UNIVERSITY KARACHI CAMPUS	2
56.	CHANGZHOU UNIVERSITY	2
57.	COMSATS UNIVERSITY	2
58.	DIRECTORATE OF AGRICULTURE RESEARCH (DATES)	2
59.	FEDERAL URDU UNIVERSITY	2
60.	FISHERIES RESEARCH AND TRAINING INSTITUTE	2
61.	ISLAMIA COLLEGE PESHAWAR	2

S. No.	Affiliations	NoP
62.	KARAKORAM INTERNATIONAL UNIVERSITY (KIU)	2
63.	KING ABDULAZIZ HOSPITAL	2
64.	KPK AGRICULTURAL UNIVERSITY PESHAWAR	2
65.	MIRPUR UNIVERSITY OF SCIENCE AND TECHNOLOGY	2
66.	PAKISTAN AGRICULTURAL RESEARCH COUNCIL	2
67.	PIR MEHR ALI SHAH ARID AGRICULTURE UNIVERSITY	2
68.	QUAID-I-AZAM UNIVERSITY ISLAMABAD	2
69.	SHAHEED BENAZIR BHUTTO UNIVERSITY OF VETERINARY AND ANIMAL SCIENCES	2
70.	SHAHEED ZULFIQAR ALI BHUTTO INSTITUTE OF SCIENCE AND TECHNOLOGY (SZABIST)	2
71.	SULTAN AGENG TIRTAYASA UNIVERSITY	2
72.	TRIBHUVAN UNIVERSITY	2
73.	UNIVERSITAS PADJADJARAN	2
74.	UNIVERSITY OF BALTISTAN	2
75.	UNIVERSITY OF GUJRAT	2
76.	UNIVERSITY OF HARIPUR	2
77.	UNIVERSITY OF KASHMIR	2
78.	UNIVERSITY OF MALAKAND	2
79.	UNIVERSITY OF OKARA	2
80.	UNIVERSITY OF SAHIWAL	2
81.	VIRTUAL UNIVERSITY OF PAKISTAN	2
82.	ABBOTTABAD UNIVERSITY OF SCIENCE AND TECHNOLOGY KP	1
83.	ABDUL WALI KHAN UNIVERSITY MARDAN	1
84.	ABDUL WALI KHAN UNIVERSITY MARDAN (AWKUM)	1
85.	ACADEMY OF PHARMACY OF PUTRA INDONESIA MALANG	1
86.	AGRICULTURAL RESEARCH COUNCIL	1
87.	AGRICULTURE RESEARCH INSTITUTION	1
88.	ASIAN INSTITUTE OF TECHNOLOGY	1
89.	AYUB AGRICULTURAL RESEARCH INSTITUTE	1
90.	AYUB AGRICULTURAL RESEARCH INSTITUTE (AARI)	1
91.	BANGLADESH INSTITUTE OF SOCIAL RESEARCH TRUST	1
92.	BEIJING TECHNOLOGY AND BUSINESS UNIVERSITY	1
93.	BINGOL UNIVERSITY	1
94.	BIO RESOURCES CONSERVATION INSTITUTE	1
95.	BOGOR AGRICULTURE INSTITUTE	1
96.	BOGOR AGRICULTURE UNIVERSITY	1
97.	BOTANICAL SCIENCES DIVISION	1
98.	BRAWIJAYA UNIVERSITY	1
99.	CANTT MILITARY HOSPITAL	1
100.	CAS CENTER FOR EXCELLENCE IN NANOSCIENCE	1
101.	CENTER FOR ADVANCED STUDIES IN ENGINEERING	1
102.	CENTRAL LUZON STATE UNIVERSITY	1
103.	CENTRE DE FORMATION ET D`APPLICATION DU MACHINISME AGRICOLE (CFAMA)	1

S. No.	Affiliations	NoP
104.	CENTRE FOR AGRICULTURE AND BIOSCIENCES INTERNATIONAL (CABI)	1
105.	CHAUDHARY CHARAN SINGH HARYANA AGRICULTURE UNIVERSITY	1
106.	CHIBA UNIVERSITY	1
107.	CHOLISTAN UNIVERSITY OF VETERINARY AND ANIMAL SCIENCES	1
108.	CHONGQING UNIVERSITY	1
109.	COMSATS INSTITUTE OF INFORMATION TECHNOLOGY	1
110.	COMSATS UNIVERSITY ISLAMABAD	1
111.	COUNCIL FOR NUTRITIONAL AND ENVIRONMENTAL MEDICINE	1
112.	DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY	1
113.	DBFZ DEUTSCHES BIOMASSEFORSCHUNGSZENTRUM GEMEINNÜTZIGE GMBH	1
114.	DE MONTFORT UNIVERSITY	1
115.	DELTA STATE UNIVERSITY	1
116.	DEPARTMENT OF BIOSCIENCES SHAHEED ZULFIKAR ALI BHUTTO INSTITUTE OF SCIENCE AND TECHNOLOGY (SZABIST)	1
117.	DEPARTMENT OF BIOTECHNOLOGY ABDUL WALI KHAN UNIVERSITY MARDAN	1
118.	DEPARTMENT OF FISHERIES FACULTY OF AGRICULTURE UNIVERSITAS GADJAH MADA	1
119.	DEPARTMENT OF HIGHER EDUCATION ARCHIVES AND LIBRARIES	1
120.	DEPARTMENT OF RESEARCH ANDDEVELOPMENT	1
121.	DISTRICT NARCOTICS CONTROL OFFICE	1
122.	DIVISION OF ANATOMY PATHOLOGY MEDICAL SCHOOL OF ANDALAS UNIVERSITY-M. DJAMIL HOSPITAL	1
123.	DIVISION OF SCIENCE AND TECHNOLOGY	1
124.	DIVISION OF SURGICAL ONCOLOGY MEDICAL SCHOOL OF ANDALAS UNIVERSITY-M. DJAMIL HOSPITAL	1
125.	ECOTOXICOLOGY RESEARCH INSTITUTE	1
126.	FACULTY OF MARINE TECHNOLOGY AND NATURAL SCIENCES OF KLAIPEDA UNIVERSITY	1
127.	FEDERAL URDU UNIVERSITY OF ARTS	1
128.	FEDERAL URDU UNIVERSITY OF SCIENCE AND TECHNOLOGY	1
129.	FOOD AND AGRICULTURE ORGANIZATION	1
130.	FOOD AND BIOTECHNOLOGY RESEARCH CENTRE	1
131.	FOOD SCIENCE RESEARCH INSTITUTE	1
132.	FORMAN CHRISTIAN COLLEGE (A CHARTERED UNIVERSITY)	1
133.	GADJAH MADA UNIVERSITY	1
134.	GC WOMEN UNIVERSITY	1
135.	GC WOMEN UNIVERSITY SIALKOT	1
136.	GHAZI UNIVERSITY	1
137.	GOVERMENT MATERNITY HOSPITAL	1
138.	GOVERNMENT COLLEGE FOR WOMEN	1
139.	GOVERNMENT COLLEGE UNIVERSITY FAISALABAD (GCUF)	1
140.	GOVT. COLLEGE OF HOME ECONOMICS	1
141.	GRADUATE SCHOOL OF UNIVERSITAS GADJAH MADA	1

S. No.	Affiliations	NoP
142.	HAMDARD UNIVERSITY KARACHI CAMPUS	1
143.	HASANUDDIN UNIVERSITY	1
144.	HAZARA UNIVERSITY MANSEHRAKP	1
145.	HENAN UNIVERSITY KAIFENG	1
146.	HORTICULTURE LAB COLLEGE OF AGRICULTURE BZU	1
147.	HUAZHONG AGRICULTURAL UNIVERSITY	1
148.	IMAM ABDULRAHMAN BIN FAISAL UNIVERSITY	1
149.	INCUBADORA EMPRESARIAL DO CENTRO DE BIOTECNOLOGIA - IECBIOT	1
150.	INDONESIAN ASSOCIATION OF BIOENERGY	1
151.	INDONESIAN INSTITUTE OF SCIENCES	1
152.	INDONESIAN INSTITUTE OF SCIENCES (LIPI)	1
153.	INDONESIAN LEGUMES AND TUBER CROPS RESEARCH INSTITUTE	1
154.	INDONESIAN LEGUMES AND TUBER CROPS RESEARCH INSTITUTES	1
155.	INDONESIAN SWEETENER AND FIBER CROPS RESEARCH INSTITUTE (ISFRI)	1
156.	INSTITUT TEKNOLOGI SEPULUH NOPEMBER	1
157.	INSTITUTE OF BEEF CATTLE RESEARCH	1
158.	INSTITUTE OF METALS RESEARCH	1
159.	INSTITUTE OF PLANT PROTECTION	1
160.	INSTITUTE OF SOUTHERN PUNJAB	1
161.	INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS (ICRISAT)	1
162.	INTERNATIONAL ISLAM UNIVERSITY MALAYSIA	1
163.	INTERNATIONAL MAIZE AND WHEAT IMPROVEMENT CENTER (CIMMYT) OFFICE	1
164.	IRRIGATION RESEARCH INSTITUTE (IRI)	1
165.	JANABADRA UNIVERSITY	1
166.	JIANGXI AGRICULTURAL UNIVERSITY	1
167.	JINNAH UNIVERSITY FOR WOMEN	1
168.	JÖNKÖPING UNIVERSITY	1
169.	KAGOSHIMA UNIVERSITY	1
170.	KAMPALA INTERNATIONAL UNIVERSITY	1
171.	KHYBER MEDICAL UNIVERSITY	1
172.	KING SAUD BIN ABDULAZIZ UNIVERSITY FOR HEALTH SCIENCES (KSAU-HS)	1
173.	KIST MEDICAL COLLEGE AND TEACHING HOSPITAL	1
174.	KOBE UNIVERSITY	1
175.	KYOTO UNIVERSITY	1
176.	KYUSHU UNIVERSITY	1
177.	LADY READING HOSPITAL	1
178.	LADY READING HOSPITAL (MTI)	1
179.	LAND RESOURCES RESEARCH INSTITUTE	1
180.	LANZHOU UNIVERSIT	1
181.	LANZHOU UNIVERSITY	1
182.	LANZHOU VETERINARY RESEARCH INSTITUTE	1

S. No.	Affiliations	NoP
183.	LIMKOKWING UNIVERSITY OF CREATIVE TECHNOLOGY	1
184.	LINNAEUS UNIVERSITY	1
185.	MERDEKA UNIVERSITY	1
186.	MUHAMMAD ALI JINNAH UNIVERSITY	1
187.	MUHAMMADIYAH MALANG UNIVERSITY	1
188.	MUHAMMADIYAH UNIVERSITY	1
189.	NAHDLATUL ULAMA SURABAYA UNIVERSITY	1
190.	NAHDLATUL ULAMA UNIVERSITY	1
191.	NAHDLATUL ULAMA UNIVERSITY OF SURABAYA	1
192.	NATIONAL AGRICULTURAL RESEARCH CENTRE	1
193.	NATIONAL COUNCIL FOR TIBB	1
194.	NATIONAL INSTITUTE FOR GENOMICS AND ADVANCED BIOTECHNOLOGY	1
195.	NATIONAL INSTITUTE OF GENOMICS AND ADVANCED BIOTECHNOLOGY	1
196.	NATIONAL UNIVERSITY OF COMPUTER AND EMERGING SCIENCES (FAST) KARACHI	1
197.	NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY (NUST)	1
198.	NED UNIVERSITY OF ENGINEERING AND TECHNOLOGY	1
199.	NEPAL ACADEMY OF SCIENCE AND TECHNOLOGY	1
200.	NORTHUMBRIA UNIVERSITY	1
201.	OCEAN UNIVERSITY OF CHINA	1
202.	PAK-AUSTRIA FACHHOCHSCHULE: INSTITUTE OF APPLIED SCIENCES AND TECHNOLOGY	1
203.	PAKISTAN AGRICULTURAL RESEARCH COUNCIL (PARC)	1
204.	PAKISTAN INSTITUTE OF NUCLEAR SCIENCE AND TECHNOLOGY	1
205.	PAKISTAN METEOROLOGICAL DEPARTMENT	1
206.	PAKISTAN UNIVERSITY OF MANAGEMENT AND TECHNOLOGY	1
207.	PARC-SOCIAL SCIENCES RESEARCH INSTITUTE	1
208.	PARC INSTITUTE FOR ADVANCED STUDIES IN AGRICULTURE	1
209.	PCSIR LABORATORIES COMPLEX	1
210.	PESHAWAR UNIVERSITY	1
211.	PHARMACY ACADEMY OF AL-FATAH	1
212.	PLANT GENETIC RESOURCES INSTITUTE	1
213.	PLANT PROTECTION RESEARCH INSTITUTE	1
214.	PMAS ARID AGRICULTURE UNIVERSITY	1
215.	PSCIR LAB COMPLEX	1
216.	PUNJAB UNIVERSITY COLLEGE OF PHARMACY	1
217.	QARSHI HERB RESEARCH CENTRE	1
218.	QUEENSLAND UNIVERSITY OF TECHNOLOGY	1
219.	REHMAN MEDICAL COLLEGE	1
220.	RESEARCH INSTITUTE FOR BIOSCIENCE AND BIOTECHNOLOGY	1
221.	RICE RESEARCH INSTITUTE	1
222.	RIPHAH INTERNATIONAL UNIVERSITY	1
223.	SAM RATULANGI UNIVERSITY	1
224.	SARHAD UNIVERSITY OF SCIENCE AND TECHNOLOGY (SUIT)	1

S. No.	Affiliations	NoP
225.	SCHOOL EDUCATION DEPARTMENT (SED)	1
226.	SEBELAS MARET UNIVERSITY	1
227.	SEPULUH NOPEMBER INSTITUTE OF TECHNOLOGY	1
228.	SHAH ABDUL LATIF UNIVERSITY	1
229.	SHAHEED BENAZIR BHUTTO UNIVERSITY OF VETERNIARY AND ANIMAL SCIENCES	1
230.	SHAHJALAL UNIVERSITY OF SCIENCE AND TECHNOLOGY	1
231.	SHENYANG ACADEMY OF AGRICULTURAL SCIENCES	1
232.	SOCIAL SCIENCES DIVISION	1
233.	THE ISLAMIA UNIVERSITY OF BAHAWALPUR	1
234.	THE UNIVERSITY OF QUEENSLAND	1
235.	THE WOMEN UNIVERSITY	1
236.	TIANJIN UNIVERSITY	1
237.	TOKYO INSTITUTE OF TECHNOLOGY	1
238.	UNIVERSITA CATTOLICA DEL SACRO CUORE	1
239.	UNIVERSITAS PRASETIYA MULYA	1
240.	UNIVERSITAS SEBELAS MARET	1
241.	UNIVERSITI KEBANGSAAN	1
242.	UNIVERSITI MALAYSIA PAHANG	1
243.	UNIVERSITY COLLEGE LONDON	1
244.	UNIVERSITY COLLEGE OF AGRICULTURE	1
245.	UNIVERSITY COLLEGE OF ENGINEERING AND TECHNOLOGY	1
246.	UNIVERSITY MEDICAL CENTER HAMBURG-EPPENDORF	1
247.	UNIVERSITY OF AGRICULTURE FAISALABAD (UAF)	1
248.	UNIVERSITY OF AGRICULTURE PESHAWAR	1
249.	UNIVERSITY OF BAHRAIN	1
250.	UNIVERSITY OF BALOCHISTAN	1
251.	UNIVERSITY OF BRITISH COLUMBIA	1
252.	UNIVERSITY OF CHINESE ACADEMY OF SCIENCES	1
253.	UNIVERSITY OF ERLANGEN-NUREMBERG	1
254.	UNIVERSITY OF FLORIDA	1
255.	UNIVERSITY OF HAMBURG	1
256.	UNIVERSITY OF HOME ECONOMICS	1
257.	UNIVERSITY OF INDONESIA	1
258.	UNIVERSITY OF JEMBER (UNEJ)	1
259.	UNIVERSITY OF LATVIA	1
260.	UNIVERSITY OF MALAKAND CHAKDARA DIR LOWER	1
261.	UNIVERSITY OF MALAKANDKP	1
262.	UNIVERSITY OF MALAYA	1
263.	UNIVERSITY OF MURCIA	1
264.	UNIVERSITY OF NORTHERN PHILIPPINES	1
265.	UNIVERSITY OF PERADENIYA	1
266.	UNIVERSITY OF PONCH	1
267.	UNIVERSITY OF READING	1

S. No.	Affiliations	NoP
268.	UNIVERSITY OF SCIENCE AND TECHNOLOGY	1
269.	UNIVERSITY OF SCIENCE AND TECHNOLOGY BANNU	1
270.	UNIVERSITY OF SINDH-JAMSHORO	1
271.	UNIVERSITY OF SOUTH AUSTRALIA	1
272.	UNIVERSITY OF SWABI	1
273.	UNIVERSITY OF THE POONCH	1
274.	UNIVERSITY OF VETERINARY SCIENCES	1
275.	WOMEN UNIVERSITY	1
276.	ZHEJIANG UNIVERSITY	1
277.	ZHEJIANG UNIVERSITY OF TECHNOLOGY	1
278.	ZHENGZHOU UNIVERSITY	1

Table 3. List of all countries with a total number of publications (NoP).

S. No.	Country	NoP
1.	PAKISTAN	167
2.	INDONESIA	32
3.	CHINA	17
4.	JAPAN	7
5.	MALAYSIA	7
6.	INDIA	4
7.	UNITED KINGDOM	4
8.	AUSTRALIA	3
9.	GERMANY	3
10.	BANGLADESH	2
11.	ITALY	2
12.	NEPAL	2
13.	PHILIPPINES	2
14.	SAUDI ARABIA	2
15.	SWEDEN	2
16.	UNITED STATES	2
17.	BAHRAIN	1
18.	BRAZIL	1
19.	CANADA	1
20.	LATVIA	1
21.	LITHUANIA	1
22.	MADAGASCAR	1
23.	NIGERIA	1
24.	NORWAY	1
25.	SPAIN	1
26.	SRI LANKA	1
27.	THAILAND	1
28.	TURKEY	1
29.	UGANDA	1
30.	UKRAINE	1

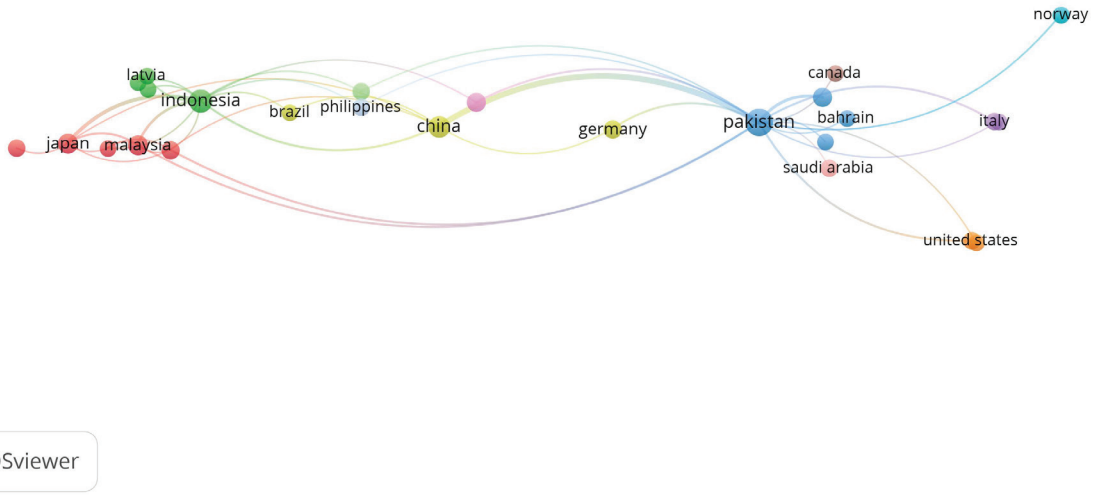

Fig. 1. Country co-authorship network (constructed on Vosviewer)

Proceedings of the Pakistan Academy of Sciences: Part B
Life and Environmental Sciences 59(2): 19-23 (2022)
Copyright © Pakistan Academy of Sciences
ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(59-2)688

Pakistan Academy of Sciences

Review Article

Application of Natural Polymers in Wound Dressings

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Abstract: A plethora of synthetic, hybrid, and biological polymers are widely being used in medical supplications. A variety of polymers are helpful in our civic activities. Their peculiar chemical, physical, and biological properties are applicable in multiple domains of life from engineering to medicine. This review specifically addresses the novel polymers and their applications in wound dressings. It has been reported by the researchers that, natural polymers are not only playing tremendous roles in micro and macro medical-industry but they are also playing a remarkable role at nano levels as nano-drug carriers and being widely used in wound dressings in pharmaceuticals. In this editorial, we will give a brief introduction of polymers and how they are widely being used in medicinal interventions, it further sheds light on the prospects of polymers with an updated version.

Keywords: Hydrogels, Natural polymers, Wound dressings.

1. INTRODUCTION

Natural polymers because of their biodegradability, biocompatibility, and closeness to the extracellular matrix are frequently used in regenerative medicine for burns and wound dressing. By triggering and stimulating the wound healing process, natural polymers aid in the restoration of damaged tissues and, as a result, skin regeneration [1]. Natural polymers have proven to be effective in a variety of biomedical applications, such as controlled administration of drugs, gene delivery, and regenerative medicine, among others. Plants, animals, and microbial are the major sources responsible for the production of natural polymers. Based on their chemistry natural polymers are divided into polysaccharide, protein, polyester, and polyamide-based polymers [2]. Because of their 3 D cross-link-based networks of polymer, immersed with water or other biological fluids, hydrogels are considered worthwhile in the Biomedical and pharmaceutical industries for wound care, burn dressing, medication administration, tissue engineering, and transplantation of organs [3].

2. NATURAL POLYMERS USED IN WOUND DRESSINGS

Here are several natural polymers which are effectively used in wound management.

2.1 Polysaccharides

There are many sources from where polysaccharides (natural polymers) can easily obtain such as gelatin, starch, cellulose, and chitosan (from vegetal source), alginate, dextran, and glucan (from microbial source) and dextran, glucan, and alginate, are all examples of animal sources. Some polysaccharides are beneficial in healing burns and wounds and are administered as hydrogels: Sulfated polysaccharides are present in different forms such as alginic acid, hyaluronic acid is acidic in nature, glucans, dextrans, and cellulose are examples of neutral sulfated polysaccharides, chitin and chitosan are basic [4]. Electrospun, dextran, cellulose, and starch are highly effective materials for producing nanofibrous composites in the regenerative medicine field (tissue engineering, wound dressing) [5]. Pullulan derivate a highly

Received: November 2021; Accepted: June 2022
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absorbent hydrogel (polysaccharides) was prepared via chemical cross-linking to use as an antiseptic wound dressing. Hydrogel is not cytotoxic and also has a significant water absorption capability (swelling ratio up to 4000 %), implying rapid hemostatic ability and preventing wound bed dryness and exudate accumulation. To treat open wounds and burns against bacterial biofilm encroachment, antibiotic and antimycotic drugs can be added to the hydrogel [6].

2.2 Glycolipids

Due to the rapid engagement of neutrophils and macrophages, topical applications of alpha Galactose liposomes comprising glycolipids with alpha-Gal epitopes could be effective in patients with wounds, burns, and other skin diseases [7]. Macrophages produce growth factors and cytokines that aid wound and burn healing by promoting re-epithelialization, tissue reshaping, and repairing processes. [10].

2.3 Proteoglycans

Synthetic or semisynthetic polymers have already been developed as biological, structural, and chemical proteoglycan alternatives. Proteoglycan is a kind of protein with covalently linked glycosaminoglycan chains [8]. Neo proteoglycan structures are biocompatible and biodegradable synthetic glycol conjugates made from protein, nanomaterial, or polymer base. They play an important role in cell-protein interactions [11].

2.4 Proteins and Peptides

Vegetal Protein: Plant-derived proteins are utilized in the treatment of wounds and burns. Soy and sodium caseinate-based membrane biomaterials have potential in drug administration and wound dressing due to their biodegradability and biocompatibility. Collagen: Collagen-based dressings are manufactured from pig, bovine, or avian sources and have been authorized for partial and packed wounds with mild - to - moderate sputum. Persons with third-degree burns (skin), as well as those who are allergic or sensitive, should avoid it [9]. Gelatin: Gelatin one of the natural polymers is a collagen-based product and is derived from a variety of by-products of animals. Gelatin is used to produce biodegradable and biocompatible drug delivery solutions and dressings for the wound in the biomedical industry. Fibrin: Fibrin is a naturally occurring scaffold for the healing of wounds as well as a cell transplant transporter. Keratin: Derivatives of keratin are utilized for the dressing of chronic wounds due to their involvement with the proteolytic wound area, which promotes the process of healing. Antibiotics and growth factors were delivered in a controllable way using new wound dressings based on keratin with improved properties [10]. Silk fibroin: Because of its unique features of biocompatibility, biodegradability, flexibility, adhesion, exudate absorption, and minimum inflammatory reaction SF is considered efficient for wound dressings. SF is also used to make tendons, prosthetic ligaments, blood arteries, and skin transplants. Enzymes: Biochemical debridement of wounds is accomplished by fibrinolytic (proteolytic) enzymes, papain, and collagenase. In pectin films effective for skin wound healing, the stability of prostaglandin H production was improved [9].

2.5 Hydrogels

Hydrogels have polar/charged functional groups that give them properties including hydrophilic nature, absorption capacity, swelling in a particular medium, and enhanced stimuli reactivity. Based on their equilibrium swelling grade, they are categorized with 20–50 percent for low swelling hydrogels (SWD), 50–90 percent for intermediate swelling hydrogels (SWD), and 90–99.5 percent for high swelling hydrogels (SWD), and more than 99.5 percent for excellent absorbent hydrogels (SWD). High Swelling grade hydrogels offer excellent biocompatibility and permeability, making them ideal for medical applications. Hybrid hydrogels are heterogeneous among hydrogels, making them ideal for adhesion of cells, organizing, and cell-cell interactions in medical applications [10]. Because of their properties, chitosan-based hydrogels are regarded as a promising choice for healing wounds, particularly major injuries. Hydrogel-based PVA/HA membranes were studied for dressings application in terms of biological properties and biocompatibility. Increasing HA components in the hybrid hydrogels resulted in decreased migration and cell viability [3]. The antibacterial efficacy of a hydrogel-based on HA/

PVPA/CS designed for cutaneous wound healing was proved against *Escherichia* coli [11]. Hybrid hydrogels based on heteropolysacchorieds used in wound dressing along with producing methods and general properties are given in Table 1.

2.6 Blended Polymers with Polyvinyl Alcohol Hydrogels for Wound Dressing

Blended polymers for medical applications were formerly defined as that interact with biological systems to assess, treat, and improve body function, or to restore any organ or tissue. Due to their intrinsic biocompatibility, biodegradable hydrogel membranes are currently being used extensively in the medical sector [7]. Sodium alginate (SA) is being investigated for use in wound dressings combined with PVA polymer as either the primary or supplemental component to the dressing structure because of its strong water swelling capacity, which impacts the local wound region beyond moisture management. PVA has exceptional capabilities of forming films and is combined with natural and synthetic polymers in the past because it is water-soluble and also contains biodegradable, biocompatible, and non-carcinogenic properties. Because it is highly hydrophilic, biocompatible, and relatively inexpensive, alginate polymer is often used in biomedical applications including scaffolds, wound dressing, and surgical or dental impression materials [8].

Table 1: In the table below, a few examples of hybrid hydrogels based on heteropolysaccharides used in wound dressing are presented, along with producing methods and general properties 4.

Hybrid Hydrogel	Producing Method	Properties
Nitrofurazone based PVA/SA hydrogels	Fourier Transform procedure	SA high concentration in PVA-based hydrogel films. Increase the ability of elasticity, swelling, and thermal stability of the PVA/SA hydrogel system. Increased SA concentration resulted in substantial reductions in the percentage of gel fraction and PVA/SA hydrogel mechanical characteristics. Reduced protein adsorption is associated with a low SA level, indicating good blood compatibility.
Clindamycin-loaded hydrogel film based Biodegradable PVA/SA (Polyvinyl Alcohol/ Sodium Alginate)	The FT technique is used to do physical crosslinking.	By increasing the SA concentration, the percentage of gelation (%), maximum strength, and break elongation of the hydrogel film reduces, also enhancing its swelling ability, elasticity, and thermal stability. The quantity of SA in the PVA/SA film does not influence the clindamycin release profile, although PVA/SA-clindamycin accelerates the process of wound healing in rats.
Alginate based PVA/calcium nanofiber web	Method of electrospinning	High calcium alginate concentration results in a high water vapor transmission rate, which aids in wound healing by keeping the immediate environment wet. Wound coated with PVA-based nanofiber; new epithelium appears to emerge without any adverse responses.

Hybrid Hydrogel	Producing Method	Properties
Hybrid hydrogels ontaining glucan		
PVA(glucan films)	Physical mixing and drying at 110 _C in the absence of chemical crosslinking	There was no covalent connection between PVA and glucan in the manufactured film; glucan can be discharged to heal faster. As the glucan content of the material rose, the tensile strength of the material dropped, while the breaking elongation increased. Cell mobility is hindered and wound healing time is increased when the PVA film contains a high glucan concentration.
Hydrogels made from chitosan (CS) and chitosan derivatives		
Hydrogel membranes based on PVA/CS (Polyvinyl Alcohol/ Chitosan)	Irradiation process follows the FT cycle	The swelling capacity, water evaporation, mechanical strength, and thermal stability of the material have all been enhanced. As the CS level rises, antibacterial action against Escherichia coli improves.
Glycerol addition into PVA/CS based hydrogels	FT after irradiation	In a rat model, the wound healing process is accelerated. L929 mouse fibroblast cells are harmless. After the 11^{th} postoperative day, mature epidermal architecture emerged.
PVA/CS hydrogel films based loaded minocycline	Fourier Transform technique	Increased CS concentration reduces the fraction of gel, mechanical properties, and PVA/CS hydrogel film thermal stability. Wounds heal faster as compared to the standard sterile gauze control.
CM/ PVA /honey (Carboxymethylated chitosan/ Polyvinyl alcohol/ honey)	Fourier Transform technique	*Escherichia coli* bacteria growth is inhibited. The honey's presence promotes wound healing.
Quaternary Chitosan/ PVA Also known as Q-chitosan mats	Photo-crosslink based electrospinning technique	Gram-positive and negative bacteria are effectively inhibited from growing.

3. CONCLUSION

Polymers, a most heard word in our daily life is a macromolecule or a large molecule, which is typically a combination of a plethora of subunits. We live in an era of the industrial revolution where we cannot imagine life without polymers. They are a salient part of our personal, domestic, and commercial life from our DNA to giant spaceships. A plethora of polymers are being used in almost every domain of medical sciences. The peculiar properties of polymers such as degree of crystallization, molecular weight adjustments, cross-linking degrees, blending abilities, biocompatibility, toxicity, tensile strengths, and characterizations make them adjustable according to the situation and desired use. An excellent wound dressing begins with careful material

selection. Biocompatibility, biodegradability, skin and environmental friendliness make naturally occurring polymers great wound dressing materials. A review of Various types of wound dressings were described in the local periodicals, each based on a naturally occurring. These Metals, antibiotics, proteins, and other bioactive compounds were included in the dressings to expedite the healing of wounds.

4. CONFLICT OF INTEREST

The authors declare no conflict on interest.

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Proceedings of the Pakistan Academy of Sciences: Part B Life and Environmental Sciences 59(2): 25-33 (2022)
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ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(59-2)718

Pakistan Academy of Sciences

Review Article

Locust Attack: Managing and Control Strategies by the Government of Pakistan

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Abstract: During outbreaks of *Schistocerca gregaria*, the desert locust swarms, and plagues known to infest numerous regions across wide areas of Asia and Africa. The locust devours large amounts of crops and rangeland flora. Recently the locust outbreak began in June 2018 in Saudi Arabia's distant areas and entered in Pakistan March 2019, destroying main crops such as cotton, wheat, rice and maize in many districts across Baluchistan, Punjab, Sindh and Khyber Pakhtunkhwa provinces. On February 1, 2020, a national emergency was proclaimed, and the Plant Protection department countered by launching monitoring and prevention activities with the help of other institutions in Pakistan, as well as global and bilateral organizations. Surveillance and control activities were carried out with the majority of the insecticide formulations being oil-based ultra-low volume, nonconventional and green technology approaches. In addition, the Pakistani government devised a comprehensive phase wised management strategy as well as a National Locust Control Center with fast retort troops deployed in critical areas. Additional surveillance and control measures are needed to stop or alleviate desert locust-related agriculture damages. The unusual characteristics of the desert locust, as well as the size and frequency of swarming events, make developing and implementing IPM measures difficult. The state of prospective integrated measures to control locusts is discussed, as well as proactive and preventive intervention options.

Keywords: Crops, Damage, Locust, Management, Pakistan, Ultra-low volume

1. INTRODUCTION

Locust, *Schistocerca gregaria* (Orthoptera: Acrididae), outbreaks have recently ravaged agricultural and natural vegetation around the world, causing tremendous damage and jeopardizing food security [1]. Due to exceptionally heavy rainfall in desert locust habitats and a lack of surveillance, political disagreements, and poor accessibility of those habitats, huge desert locust plagues and swarms travel over the Arab World, Central Africa, India, and Pakistan. Despite technological advancements in locust monitoring, prediction and management measures, the threat and harm induced by locust pests are still prevalent, affecting hundreds of millions of human lives. Desert locust is the old transborder insect pest that destroys practically all fields and rangeland plants in wide parts of Asia and Africa [2].

The migratory pest undergoes 'phase change' from solitary withdrawal to sociable cluster behaviour in reaction to environmental conditions linked with extensive vegetation flushes that sustain considerably increased desert locusts numbers and densities become crowded when water becomes foliage fades and limited [3], a pheromone-modulated transition from locust's lonely phase to physically and behaviorally different sociable stage enabling grouping into nymphal bands and adult groups occur [4]. Gregarized desert locust occurrences have occurred intermittently during the previous two millennia, usually originating in known breeding regions the majority of the

Received: April 2022; Accepted: June 2022
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important breeding grounds are in isolated, rugged terrain which is frequently plagued by instability [3].

The 2003–2005 desert locust outbreak impacted more than eight million people in twenty countries [5] resulting in a projected yield loss of million $2.5 [1]. Pakistan lies within the geographical distribution of the desert locust, but the repercussions of locust activity there have not been documented in journalism until now. This study is to explain the timeline, feedback, and consequences of the latest locust outbreak caused in Pakistan, as well as food security threats and their further strategies to control locusts through their early approaches of IPM and green technology.

2. LIFE CYCLE

Grasshoppers are found in tropical, moderate grassland, and desert habitats and number around 10,000 species. Locusts are a category of 18–21 species that can fly across distant locations and are one of the first migratory pests to affect crops. Desert locusts (*S. gregaria*) are the most common locust species, with roughly ten subspecies found in Europe, Asia, Africa, and Arab countries [6]. These are the greatest and mainly dangerous insect groups when it comes to crops and food sources [7].

Locust is a major issue due to its capacity to traverse vast rang and quick grow populations, although the migratory locust, *Locusta migratoria* is the most common species [8]. Female locust life cycle ranges from 3 to 6 months and she can lay roughly 100 eggs every day. The larvae grow quickly, maturing in around 20 days [9]. Temperatures 25 - 32°C with humidity of 85-92 % and soil humidity of 15-18 % are ideal for locust growth [10]. Drought diminishes vegetative wrap, which shields the eggs until rainy circumstances are favorable for spawning [11]. The Supplementary Information contains more information about locust biology.

The enormous movement of locusts is triggered by changes in neurotransmitter serotonin levels [12]. Hyperpolarization suppresses the leg muscle fibers' post-synaptic potentials, weakening the depolarized excitatory post-synaptic response and enabling the locust to jump one hundred times its body length [13]. Locusts can fly constantly for over 10 hours a day, covering distances of approximately 150 km [14]. In comparison to other species, the

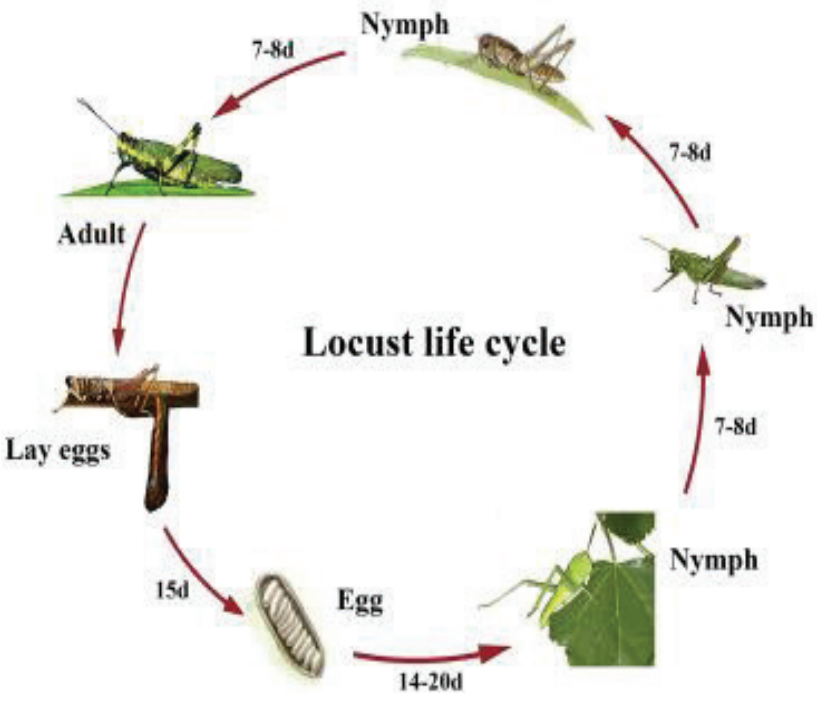

Fig. 1. Life cycle of locust

desert locust may replicate a single generation in less than a month, which is six times more rapid than other locust species [15]. The individuals can live up to three months, which, along with their rapid expansion, results in a 20-fold increase in population every generation [16].

3. SOUTH ASIA'S RECENT DESERT LOCUST ACTIVITY: A REGIONAL PERSPECTIVE

Asia encompasses Afghanistan, Bangladesh, India, Iran, Sri Lanka, and Pakistan as significant agricultural countries, with the majority of the people, engaged in subsistence agriculture [17]. The Thar Desert, along with the southern sections of the India–Pakistan border, is a prominent desert locust breeding location in Asia. Between 1947 and 2021, desert locust behavior along the India–Pakistan border was not constantly evaluated, and outbreaks were not managed [18]. From 2012 through the most current outbreak (2018–2021) in South Asia, a minimum of five noteworthy locust outbreaks occurred [19]. The latest upsurge originated in the Arabs' distant areas and arrived in Iran, Africa, and Yemen in June 2019, damaging millions of tons of crop production. Swarms of locusts infiltrated Pakistan from Iran and began breeding there due to favorable climatic circumstances [20-21]. The plague was the worst upsurge of locusts affecting many regions, during the years 2020–2021, the crisis in South Asia deepened affecting many countries including Pakistan [3].

4. DESERT LOCUST ACTIVITY IN PAKISTAN

Pakistan has been affected by significant desert locust swarms four times in the previous 100 years: 1926, 1952, 1962, and 1992 [22]. Due to conflicting needs, Pakistan's preparation worsened during the interval, as the 1992 attack happened 27 years until the latest incident. In the latest occurrence, Pakistan's Space and Upper Atmosphere Studies Commission identified locust susceptible zones based on plant communities, type of soil, history, or environmental circumstances [23]. About 36.9 % of Pakistan has been identified as being vulnerable to locust plague [24].

Swarms that formed in Saudi Arabia started spreading west over Africa, Yemen, and east to Iran by late 2018. Swarms had spread to Pakistan by March 2019, as well as Kenya and Uganda in East Africa [25]. By June, locusts had infested crop fields in different districts of Baluchistan in Pakistan [26] and had damaged cotton and other vegetable crops in different districts of Sindh Province. The damages and many crop losses have been occurred and reported in Punjab, Sindh, and Baluchistan [27]. The gregarious feeder's locust widens in different regions of Cholistan, Nara, and Thar deserts along the Pakistan-India border in mid of 2019, damaging 68,000 km^2 [28] and damaging 33 % of field crops [29]. Locust plagued different areas of the Korangi and Malir districts of Karachi [30]. Locust movements increased dramatically on the sides of desert borders during the May-June rains [31].

5. IMPACTS OF LOCUST DAMAGE

Crop damage by locusts compounded the negative consequences of two to three years of famine and an increasing situation of declining water supply [32]. In addition, the country has had 12 years of significant financial rise; the sugar price, such as, had increased, while wheat edible prices jumped 15% in 2020 [33]. Mangoes, potatoes, cotton, cumin, and fodder grasses were all hit hard by swarms [34]. In wheat, chickpea, and potatoes serious attacks and crop losses of about 15 percent occurred estimated at approximately US $1.2 billion [23].

During the winter season losses were estimated to be US $2.2 billion and US $2.9 billion for the Kharif season at a theoretical 25 percent damage [23]. Natural calamities and harsh environments, for example, famine, floods, and high saline soil afflicted the most susceptible areas, contributing to food insecurity. Due to harsh circumstances, the Khyber Pakhtunkhwa government declared agricultural emergencies in 35 districts [31]. To diminish agricultural dangers caused by locusts, a national emergency was stated on February 1, 2020 [1]. The National Disaster Management Authority of Pakistan recorded locust swarms in 61 districts across the country.

6. PAKISTAN'S GOVERNMENT ADOPTED STRATEGIES

To combat the current desert locust outbreak, Pakistan's government adopted proactive measures in coordination with the United Nations' Food and Agriculture Organization and neighboring nations' aid agency partners. In February 2020, Pakistan's cabinet approved a brief three-phased National Action Plan for locust management which comprises threat measurement scenarios and set up of surveillance and control efforts connected to dates of cultivation and the utilization of agricultural land [35]. Through surveillance and control, Phase 1 aims to restrict the intensity and growth of desert locust populations while extenuating human and ecological concerns. This entailed obtaining climate data in high-risk areas to enhance target control efforts. By April 26, 2020, 76.9 percent of Pakistan's land had been surveyed and 5.5 percent, had been implicated with pesticides [33].

During the second phase, which ran from July 2020 to December 2020, government make a strategy to protect crops that were connected to domestic and overall locust monitoring and control networks, providing instant aid to growers and livestock owners and strengthening national capability for advance caution and interference [35]. The Ministry of National Food Security and Research's power to control desert locusts was further bolstered by the improvement of Food Security and Nutrition Information [36].

During the 3rd phase, the government allocated $76.1 million of funds to the economic year 2021 to boost Pakistan’s Project Management Unit’s ability to plan accomplishments and surveillances to create desert-free regions from locusts, which began after December 2020 [36].

6.1 Chemical Measures

Most insecticides used in control activities were conventional synthetic insecticides, with accessibility, protection, cost-efficient, and environmentally compatible influencing insecticide selection and application timing [38]. Diflubenzuron, IGR is one of the most extensively used as a substitute for traditional pesticides as IGRs prevent arthropods from molting, and vertebrates are unaffected [39-40]. The majority of pesticides used against locusts were made with ultra-low volume oil-based formulations administered with special spray equipment [40].

6.2 Integrated Pest Management (IPM)

To accomplish ecologically friendly and long-term pest management, Pakistan's government created a National Integrated Pest Management Plan. While reducing or eliminating the danger of insecticide resistance is one of the aims of IPM [41]. Locust epidemics are infrequently enough already to prevent this. Desert locust resistance to any of the insecticides used against them in natural conditions has not been reported, so IPM will concentrate on facilitating effective interventions, locust control economics (including insecticide costs), and implementation of increasingly ecologically sound measures and pesticide manager and sprayer protection.

In Pakistan, no pesticides used to combat desert locusts are classified as class A (extremely hazardous) or class B (very dangerous), according to the WHO. If low-toxicity techniques are employed, human intoxication from eating sprayed locusts could be minimized as well. This may also help to decrease the accumulation of pesticide containers that are occasionally used to keep drinking water and eating food for humans and farm animals without being decontaminated [42].

Because of the particular nature of locust biology, upsurges, and management, a flexible solution based on IPM-compatible components is required, Although, biopesticides have been used to control swarming locust species in Pakistan and they have not been well assessed under outbreak settings in Somalia [43]. In comparison to typical synthetic pesticides, they are temporary and degrade as the temperature increases. Suspicions about human and environmental safety are another impediment to biopesticide application and many continents have uttered concern that entomopathogen isolates discovered in one locust-affected region will cause an exotic risk to other nearby countries.

The most promising and available method at the moment is intervention timing, which focuses on the early stages of gregarization. Extensive

control in reproduction regions from a single pesticide treatment on foliage and crops is not a viable tactic because long-term, broad-spectrum organ chlorinated insecticides have wide residues in the environment and broad-spectrum in nature and have not been in use since the 1980s [3].

The intervention of low residual pesticides is planned with higher accuracy to provide early detection needing monitoring that is effective enough to identify the start of phase transition. As a result, monitoring technologies must be able to track desert locust activity across time, both during and between episodes. This entails analyzing geo-historical locust activity and control as well as using GIS imagery and meteorological data [44] to get an absolute understanding of propagation within Pakistan.

Integrations of locust density, food supply abundance, or clustering which can signify the start of gregarization in locust-favorable combinations, are likely to be used as intervention thresholds [45]. Further tools for detecting the onset of gregarious behaviour to timely intervention such as sensors for semiochemicals related to phase transformation in desert locusts, may be developed in the future [3]. Due to disparities between different environments, monitoring population density alone is unlikely to be sufficient for projecting gregarization [44].

Climate patterns determine rainfall levels, wind speed, and direction are markers that influence mating and swarms' migration, affecting early intervention thresholds. Although no effects on solitary desert locusts were observed, Pakistan could help from the study that revealed a swarm displacement cycle in Northwest Africa. According to a swarm displacement cycle study, soil dampness persistence in low-lying places after rainfall promotes the growth of green vegetation, which provides food for desert locusts. Phase transformation anticipation, which has improved control in Morocco [46] can be used in Pakistan, especially when synchronized with India. Other characteristics of spatiotemporal desert locust aggregation patterns will aid in the streamlining of surveillance efforts to trigger early action. Nymphal bands, for example, avoid particular plants and sleep in clumps on sparsely scattered trees and huge shrubs at dusk. Also, when temperatures rise over a certain threshold for flight, swarms of insects search for shelter in the covering interiors of small bushes but do not those rest in giant plants [47]. Such findings can be included in surveillance efforts in Pakistan aimed at establishing the best time to intervene early in the case of desert locusts.

6.3 Green Strategies

Hazardous pesticides should be replaced with green solutions in locust management since pesticides are harmful to human wellness and have longer consequences for wildlife habitats [48]. Microorganisms that are fungus and bacteria, for example, have been used successfully in combination with harmful plant species in innovative biological pest control [49]. Wasp larvae, mites, spiders, and birds, for example, can reduce epidemics by up to 90% by predating larvae and growing locusts [50].

It is critical to conserve these natural enemies' ecosystems and biotopes, for example, through tropical forests [51] which, when intercropped with late-growing crops like alfalfa and fruit trees are effective measures [52]. Additional effective mitigating measures include achieving more than 50 percent foliage coverage by using locust-toxic and wild plants in conjunction with animal husbandry feeding strategies [53]. Converting lowland areas into fishponds and shrimp farms as part of the landscape shapes a different viable way to manage locust upsurge [54]. Meanwhile, the burning of plants and lighting of bonfires in the dark are effective to control locusts [55-56].

Physical traps and optical and mechanical devices were used to limit plague outbreaks in risk-bearing areas. When combined with exact Bayesian prediction modeling, these traps provide a cost-effective and long-term solution [57]. Because locusts have phototaxy, stimulating them with specific light and sound wave lengths improves trapping by interacting with their eyesight and sound via glutamate and dopamine neurophysiology [58].

Remote sensing is also being used to predict locust eruptions based on habitat greenness, which seems to be a more viable approach than typical satellite and radar data [59]. These technologies are not fully developed in general, and they will require central government support before they can

be used on a broad scale for management and cure [60]. Supplementary Information contains more information about locust control.

6.4 Future Strategies for Locusts Controlling

There are currently two methods for controlling desert locusts. The first, reaction, is a standard response to outbreaks, upsurges, and plagues that pose immediate threats to agricultural production. Pro-action is the recommended technique, which comprises intervening while outbreaks are in the early stages of development, reducing them before they become epidemics. Both techniques rely on synchronization, but the reaction is haphazard and not acknowledged as a well-thought-out strategy [61]. Pro-action on the other hand has decreased the number of important occurrences by remaining ready to combat gregarious locust populations, whether they arise inside or beyond the regions [62].

The best managing technique is prevention, which is intervening early enough to prevent epidemics from occurring in the first place. While there is no way to avoid outbreaks at this time, continuous study and coordination efforts could lead to the discovery of a way to keep locust populations in their lonely phase indefinitely. Preventive skills will require fully efficient collaboration at international, regional, domestic, and local dimensions to impose timely control in breeding areas [61]. Although the majority of major outbreaks have occurred outside of Pakistan [3] Pakistan must be ready to mobilize people and material resources to confront swarms from neighboring countries as well as epidemics in the deserts along the Pakistan-India border. The National Locust Control Center was formed during the most recent outbreak to collect statistics on desert locust activity, which affected more than 38 % of the country [63].

Increased precipitation in South Asia is expected as the seas and oceans warm which increases the incidence of Indian Ocean Dipole-influenced weather-related events around the world [64]. As a result, it is expected that the intensity and frequency of episodes to increase. These potential highlights the necessity for governments to work together on a global level to improve proactive interference [3]. Each locust-swarmed country must maintain desert locust management capabilities throughout recessions and possibly integrate effective control strategies to meet human and ecological safety concerns.

7. CONCLUSION

From 1912 to 2020, there have been seven significant locust outbreaks that have all been linked to prolonged droughts, mild winters, and the occurrence of substantial spring and summer precipitation. The agricultural industries, which are the cornerstone of national economies and social stability, are severely affected by the pandemic. To reduce outbreaks, environmentally friendly alternatives to hazardous pesticides must be developed. The utilization of microbes, insects, and birds in biological control approaches helps to manage outbreaks while minimizing the harm that pesticide use does to the environment and agriculture. Additionally, reforestation of arable land reduces local climatic changes, leading to reduced temperatures and less precipitation, while also attracting more birds, which raises the predation rates of locusts. Although green technologies like light and sound stimulation appear to be effective, they are difficult to use and require additional technological advancement, particularly the integration of remote and modeling, before they can be used on a large scale.

8. CONFLICT OF INTEREST

The authors declare no conflict on interest.

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Proceedings of the Pakistan Academy of Sciences: Part B
Life and Environmental Sciences 59(2): 35-47 (2022)
Copyright © Pakistan Academy of Sciences
ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(59-2)707

Pakistan Academy of Sciences

Research Article

Phenotypic Variability and Resource Allocation in Kashmir Sage (*Phlomis cashmeriana* Royle ex Benth.) in relation to Different Habitats and Altitudes

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Abstract: *Phlomis cashmeriana* Royle ex Benth. commonly known as Kashmir sage is a rare and important medicinal plant growing in Kashmir Himalaya. The current study is the first of its kind, carried out to find the impingement on growth dynamics of the plant under study along various altitudinal inclines and habitats. Extensive field surveys were conducted during the years 2020-2021 to assess the distribution and phenotypic attributes of *Phlomis cashmeriana*. Three natural sites viz, Jawahar tunnel, Hillar naar, Daksum and one control population at KUBG were selected for the present work. Morphological characteristics of species were noticed to be varying extensively under different scopes of environment. Plants thriving at lower altitudes (KUBG) were found to be growing vigorously and taller. However, the opposite scenario was observed in plants growing at higher altitudes (Jawahar tunnel). KUBG and Daksum were reasonably found to be better habitats for the growth of *Phlomis cashmeriana* as revealed by principal component analysis (PCA). Majority of resources were attributed towards the growth of rhizomes followed by leaf, stem, and inflorescences respectively. Variation in reproductive success was also observed along different altitudes ranging from 63.94 % to 53.40 %. The total resource budget per plant also varied among different populations with a maximum in populations growing at low altitude, KUBG (23.73±6.63) and least in populations growing at high altitude J. tunnel (12.94±7.67). We hypothesize that a heterogeneous environment is the primary cause of phenotypic variability across different altitudes, however, the role of other environmental factors should also be taken into consideration.

Keywords: Phenotypic Variability, *Phlomis cashmeriana*, Resource Allocation, Reproductive Success

1. INTRODUCTION

Sessile nature of plants has made it obligatory for them to make fine adjustments genetically or morphologically to varying environmental conditions through phenotypic plasticity within a geographical area [1]. In the present study, we evaluated the information on plant plasticity under changing environmental conditions within a geographical area along different altitudinal gradients. Despite receiving huge attention from ecologists as well as evolutionary biologists the research on the study of phenotypic plasticity and its implication in the changing environment is largely obscure [2]. Quantification of changes involved in the physiognomy of plants on different steep environmental gradients like altitude is rarely being done besides being evident. These changes have resulted in the distortion of the pattern involved in the assimilate investments and thereby affecting the leaf microclimate to which they are exposed [3]. Considering higher altitudes, dramatic variations in abiotic factors take place over small distances, contributing to key

Received: March 2022; Accepted: June 2022
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alterations in selection pressures acting on different plant traits [4]. Among different plant species, the response to these selection pressures is significantly varied. So far altitudinal gradients are reckoned they provide profuse experimental prospects for looking into the functional traits accompanying these plants to withstand the implication of the changing environmental conditions [5]. In stressful conditions, for perseverance plants allow morphological and physiological adjustments at higher altitudes such features include dwarfness, habit compaction, small and densely pubescent leaves. In addition to this, physiology of the leaf also changes with altitude [6]. Population divergence is tempted by selection pressure differences forced by different neutral evolutionary processes and ecological environments or both. The change in the phenotypic characters plays an important role in the reproductive behavior of specific plant species [7]. *Phlomis cashmeriana* (Lamiaceae) commonly known as Kashmir sage belongs to the family Lamiaceae and is often found growing in wastelands or on open slopes and blooms in summer [8]. *P. cashmeriana* is a perennial herb native to Afghanistan, Pakistan, Tadzhikistan and the west Himalaya [9]. It has several stems (40-80 cm), simple or branched with a sturdy woody rootstock. Leaves are oblong-lanceolate, leathery, covered with densely whitish trichomes, Inflorescence verticillasters, flowers labiates, and corolla lobes pale purple. Species of this genus have been used for many decades in folk medicine as tonics, wound healers, and stimulants [10]. Some biological and pharmacological activities such as antidiabetic [11], antiulcerogenic, antimicrobial [12], anti-inflammatory, antinociceptive, antifibriel, and immuno-suppressive [13] have also been documented.

2. MATERIALS AND METHODS

2.1 Study Area

The current study was carried out during 2020-2021 across Kashmir Himalaya, India. Extensive and robust field surveys were conducted to assess the distribution and phenotypic attributes of *Phlomis cashmeriana*. It was found growing across diverse habitats, altitudes and areas of Kashmir including (Hillar Naar, Sehpora Dooru Anantnag, Jawahar tunnel, Daksum vailoo, Koot Larnoo, Shatroo, Soaf Shali Kokernag, Adigam Kokernag, Deral Gam Kokernag, Panzgam kokernag, Dandipora Kokernag). Three natural sites viz, Jawahar tunnel, Hillar naar, Daksum were selected based on availability, accessibility and abundance of the population to carry out the present work Besides, one established population at Kashmir University Botanical Garden (KUBG) was taken as control (Figure 1). The characteristic features of selected sites and geo-coordinates (using Garmin GPS etrex 10) were recorded (Table 1). Herbarium specimens from selected sites were submitted in KASH Herbarium under voucher specimens No. 2941, 2942 & 2943). The distribution map and map of selected sites of *P. cashmeriana* is shown in Figure 2 and 3 respectively.

2.2 Morphological Characterization

The research was carried out in three natural habitats and one controlled site (*ex situ* and *in situ*). images were shot with MI note 8 pro camera having a resolution of 64 mega pixels. To observe different morphological features of the species and to record the variability in floral and vegetative properties, twenty full flowering individuals were randomly selected and tagged from each group. Most of

Table 1. Salient features and geo-coordinates of selected study sites for the collection of *P. cashmeriana.*

Study site	Altitude (m-asl)	Latitude and longitude	Climatic zone	Habitat
Jawahar tunnel	2580	33°6'N,75°2' E	Temperate	Sunny open Rocky slope
Hillar Naar	2200	33°5'N,75°18' E	Temperate	Sunny open Rocky slope
Daksum	2000	33°5'N,75°3' E	Temperate	Sunny with Partial shade
KUBG	1595	34°5'N ,74°48' E	Temperate	Moist open field

Fig. 1. Different study sites of *P. cashmeriana*: A) Daksum, B) Hillar Naar, C) KUBG, D) Jawahar tunnel

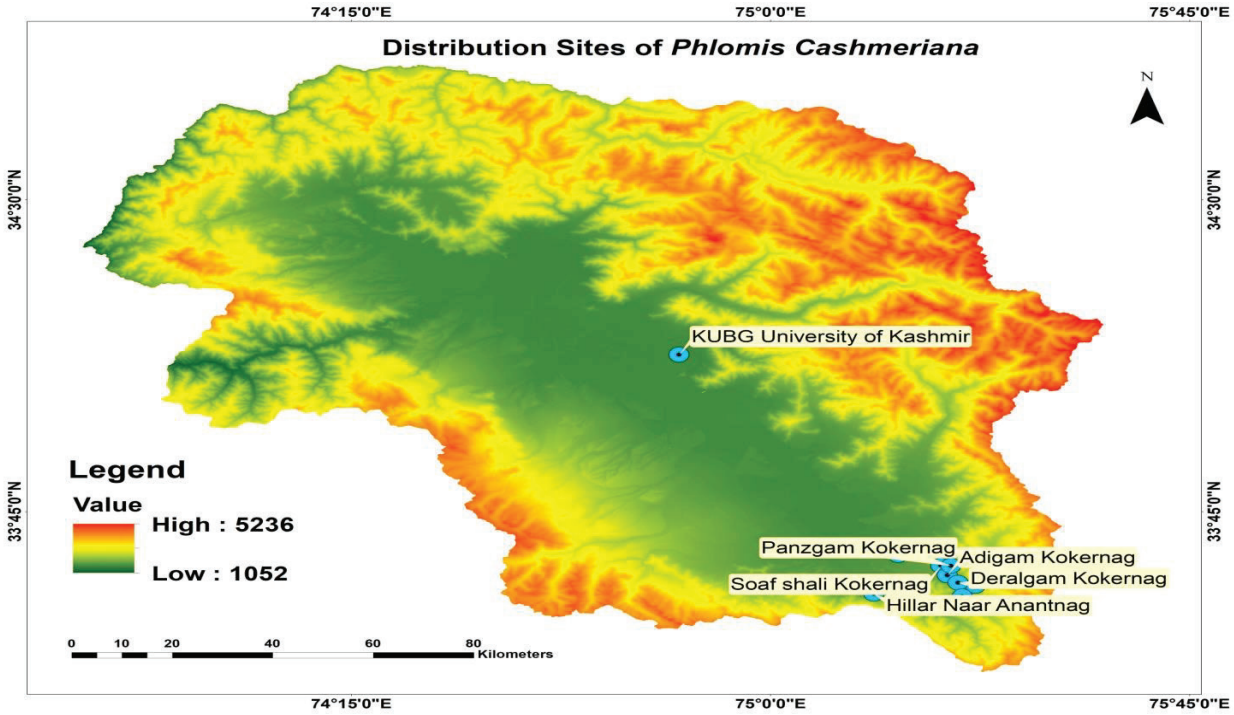

Fig. 2. Distribution map (Asterdem) of *P. cashmeriana*

these characteristics were recorded after the plants were measured *in situ*. All populations were at the flowering stage at the time of collection. To locate the places of improved growth performance for this species, we performed linear regression analysis to establish the link between several morphological features across different altitudes. The morphological features were analyzed using principal component analysis (PCA) with habitat dynamics. It was also used to determine the degree of coherence between several vegetative and reproductive indicators.

2.3 Resource Allocation

Resource allocation pattern in different plant parts was determined by harvesting twenty matures plants from each selected study site. The harvested plant material was partitioned into different parts (Rhizome, Stem, Leaf, and Inflorescence). Fresh weight of every individual plant part from each plant was measured using an electronic weighing balance. After measuring the fresh weight of every plant sample, the plant material was oven-dried using a hot air oven (72 hours at 80 °C) then the dry weight of the plant material was determined following Kawano and Masuda with slight modification [14]. Reproductive effort (RE) was calculated from the estimates of dry weight or biomass allocated to reproductive and vegetative structures following [15,16]

$$\text{Reproductive effort} = \frac{\text{Dry weight of the inflorescence}}{\text{Total dry weight of the above-ground tissues}} \times 100$$

2.4 Reproductive Success

Flower heads were collected from each selected population during the peak flowering season. The number of flowers per head was counted, and the dimension of floral parts includes sepal breadth and length; upper lip (petal) length and breadth; stamen and carpel length and breadth were measured to examine the proportion of variation within and among plants as well as among populations. We determined the percentage seed set of each plant expressed as the ratio of total no. seeds produced to the total no of ovules borne by a plant. Developed seeds and undeveloped seeds were easily distinguished because the developed ones were plump and hard while undeveloped ones were hollow and soft [17].

2.5 Data Analysis

SPSS software (version 20.0) was used to carry out ANOVA to check the difference between different morphological attributes. Tukey's multiple comparisons of means were also used to compare different populations and variations within the individual as well as average means, means were considered to be significant at ≤ 0.05. To carry out linear regression analysis Origin 2021 was used to find out the correlation between different morphological characters along different altitudes. Analysis between different morphological characters with respect to different habitats and to understand the coherence between different vegetative and reproductive parameters principal component analysis (PCA) was also carried out.

3. RESULTS

3.1 Morphological Characterization

During the present study it was found that *P. cashmeriana* prefers open rocky slopes (Table 1). *P. cashmeriana* is an erect, densely woolly perennial herb with a sturdy woody rootstock, stems several, 40-80cm herbaceous, simple or branched, with a white indumentum of readily rubbed off dendroid-stellate hairs. Leaves are oblong-lanceolate, densely white tomentose beneath with dendroid-stellate hairs, above green with scattered irregularly branched dendroid hairs (Figure 4).

The present study revealed a clear-cut difference in morphological traits along different altitudes and habitats (Table 2). It was found that the plant height is maximum at low altitude, KUBG (58.71±13.53 cm) while minimum height was seen in plants growing at Jawahar tunnel (40.6±6.09 cm). Root length and breadth were maximum in plants grown at KUBG (27.5±6.57 & 1.97±0.38 cm) while as a minimum at Jawahar tunnel (12.02±3.02 & 1.53±0.32 cm). Much difference wasn't seen in the number of leaves; maximum leaf no was seen in plants growing at Daksum (25.7±6.68 cm) while a minimum number of leaves were present in plants growing at J. tunnel (18.9±5.93 cm). Maximum variation was seen in basal leaf length and basal petiole length, the plants grown at low altitude

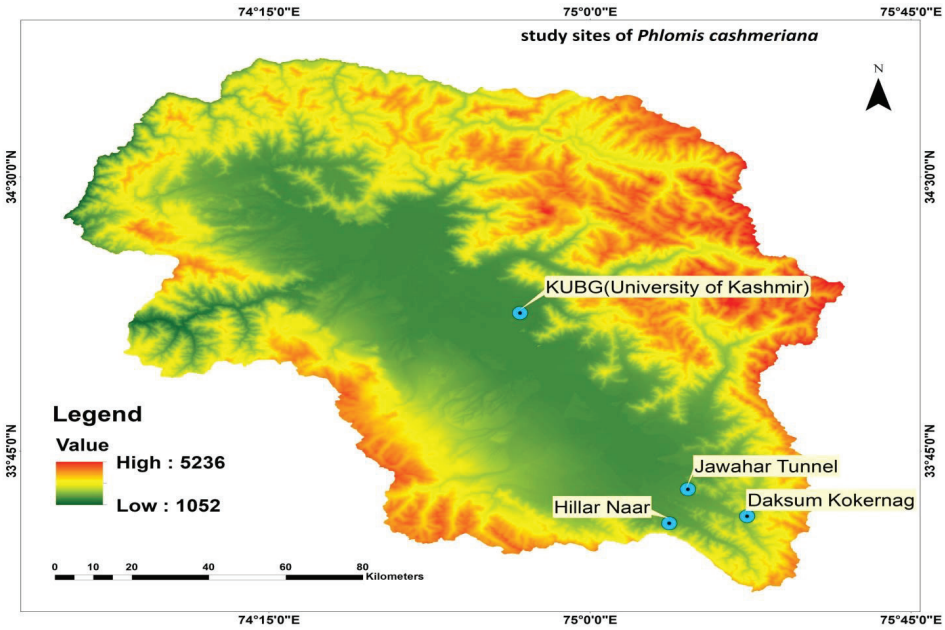

Fig. 3. Location map (Asterdem) of study sites

Fig. 4. (A-F). Morphological features of *P. cashmeriana* (A) Habitat, (B) Rhizome, (C) leaf with petiole abaxial and adaxial view, (D) Inflorescence, (E) Flower, (F) Seeds

Table 2. Variation in different morphological traits (Mean±SD) of *P. cashmeriana* across different study sites (2020-2021)

Plant characteristics	Jawahar tunnel (cm)	Hillar Naar (cm)	Daksum (cm)	KUBG (cm)	*F* value
Plant height	40.6±6.09^{c*}	50.8±3.49^{ab}	53.62±12.80^{ab}	58.71±13.53^{a}	8.027
Rhizome length	12.02±3.02^{b}	16.15±8.64^{a}	19.02±1.76^{b}	27.5±6.57^{a}	9.43
Rhizome breadth	1.53±0.32^{a}	1.67±0.69^{a}	1.8±0.76^{a}	1.97±0.38^{a}	0.651
No. of leaves	18.9±5.93^{a}	19.5±9.00^{a}	25.7±6.68a	23.43±12.50^{a}	1.847
Basal leaf length	9.953±2.65^{b}	11.58±1.05^{ab}	12.62±2.80^{a}	17.22±4.05^{a}	7.789
Basal leaf breadth	3.31±0.40^{b}	3.84±0.50^{a}	4.03±0.48^{a}	5.51±0.97^{a}	6.534
Basal Petiole length	7.48±1.55^{b}	8.43±2.70^{a}	9.16±2.43^{a}	13.78±1.82^{a}	9.231
Apical petiole Length	0.87±0.247^{c}	1.04±0.20^{bc}	1.38±0.36^{ab}	1.56±0.17^{a}	12.295
Apical leaf length	5.75±0.58^{c}	6.78±1.4^{b}	7.60±1.10^{ab}	10.36±0.97^{a}	15.934
Apical leaf breadth	1.87±0.40^{c}	2.18±0.39^{b}	2.47±0.20^{ab}	3.05±0.46^{a}	15.271
Inflorescence length	7.91±3.99^{ab}	6.68±5.54^{a}	13±7.35^{a}	8.42±5.40^{a}	2.820

* Means labeled with the different small letters indicate that they significantly differ from each other among different populations

KUBG show almost twice the length (17.22±4.05 and 13.78±1.82 cm) as compared to plants grown at higher altitude Jawahar tunnel (9.953±2.65 and 7.48±1.55 cm). Maximum number of flowers and flower heads per plant at KUBG was maximum (18.28±3.81 & 4±1.67 cm) respectively while the minimum number was seen in plants growing at Jawahar tunnel (16.6±4.47 & 3.3±1.95 cm) (Table 3). Inflorescence length was maximum in individuals growing at KUBG (8.42±5.40 cm) while a minimum in plants growing at Jawahar tunnel (7.91±3.99 cm). Positive correlation was observed (Figure 5 & 6) between plant height and rhizome breadth (r^2 = 0.01187), plant height and rhizome length (r^2 = 0.10595), plant height and basal leaf length (r^2 =0.11591), plant height and basal leaf breadth (r^2 =0.23675), plant height and apical leaf length (r^2 = 0.22617), plant height and apical leaf breadth (r^2 = 0.43063), plant height and basal petiole length (r^2 = 0.07359), plant height and apical petiole length (r^2 = 0.09965), plant height and number of leaves (r^2 = 0.06787) , plant height and inflorescence length (r^2 = 0.08214), plant height and flower no. (r^2 = 0.00376)

Principal component analysis (PCA) of all morphological characters across the study sites reveals that the major differences between populations were due to size characteristics (axis 1; 91 % of total variance) and separated KUBG and Daksum (with high character values) from the other populations (Figure 7). PCA also revealed that the high altitude populations are non-favorable to most of the vegetative and reproductive traits demonstrating better growth conditions at lower altitudes. Apical leaf length, Apical leaf breadth, Rhizome length, Basal leaf length, Basal leaf breadth, and Basal petiole length were found to be favoring the KUBG population. Leaf number, Apical petiole length, Rhizome breadth, and Plant height were found to be favoring the Daksum population. Thus, the habitat of KUBG and Daksum proved relatively better for the growth of *P. cashmeriana.*

3.2 Resource Allocation

Resource portioning in various parts of plants varies differently as shown in Table 4. The population under study shows great variation in the overall dry weight of the above-ground and dry weight of different vegetative structures of plants growing

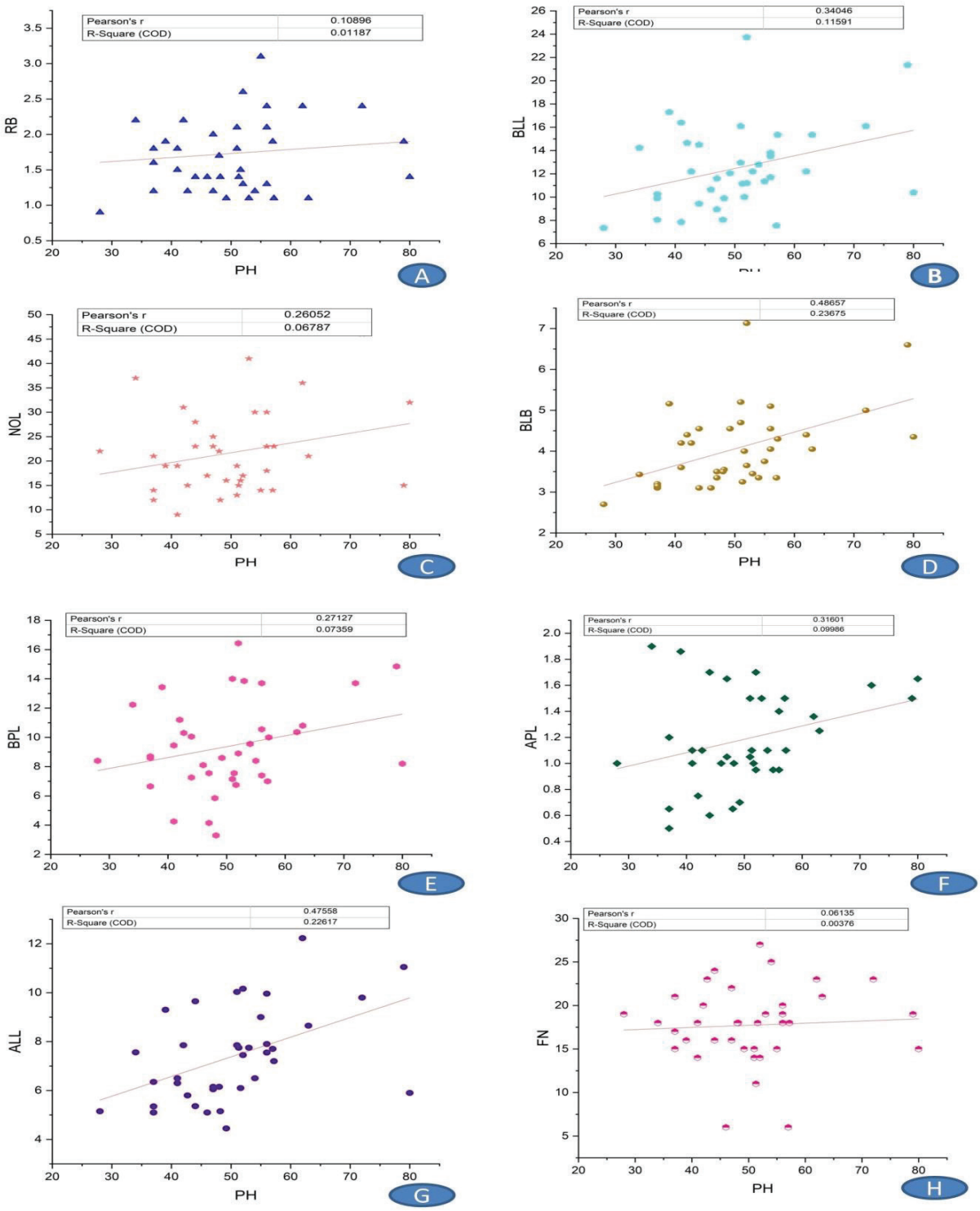

Fig. 5. (A-H) Regression analysis between several morphological features of *P. cashmeriana*

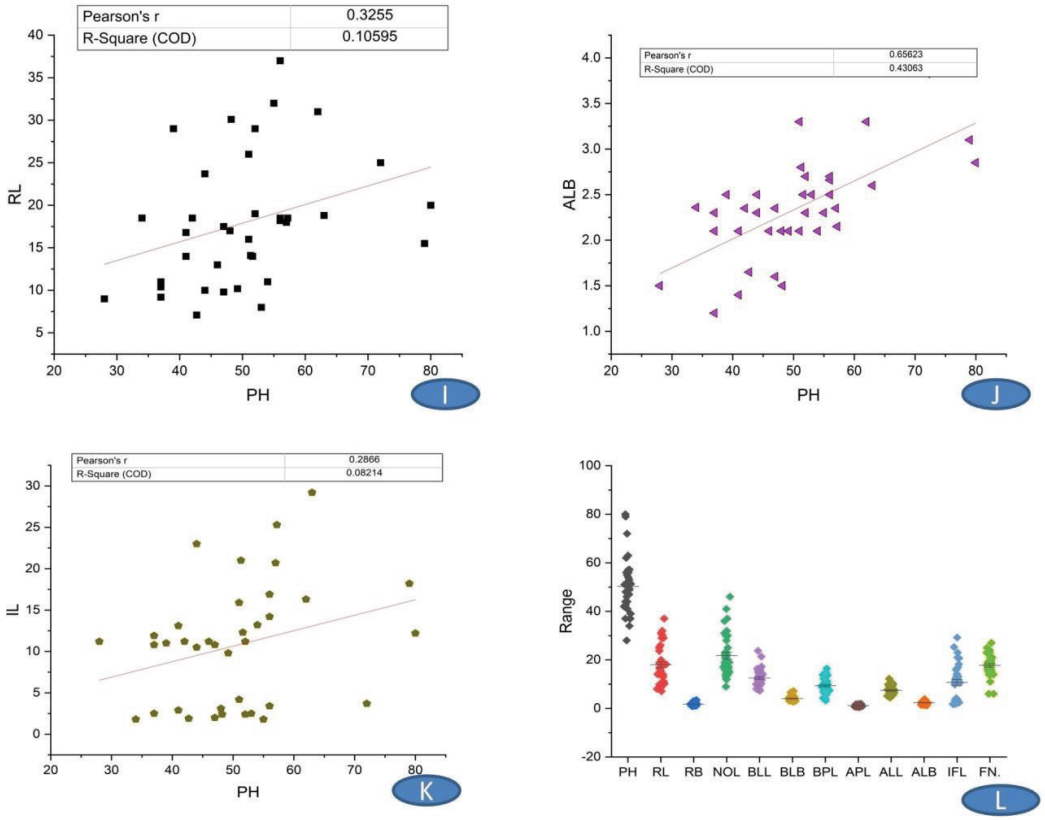

Fig. 6. (I-k) Regression analysis, (L) Range between several morphological features of *P.cashmeriana*

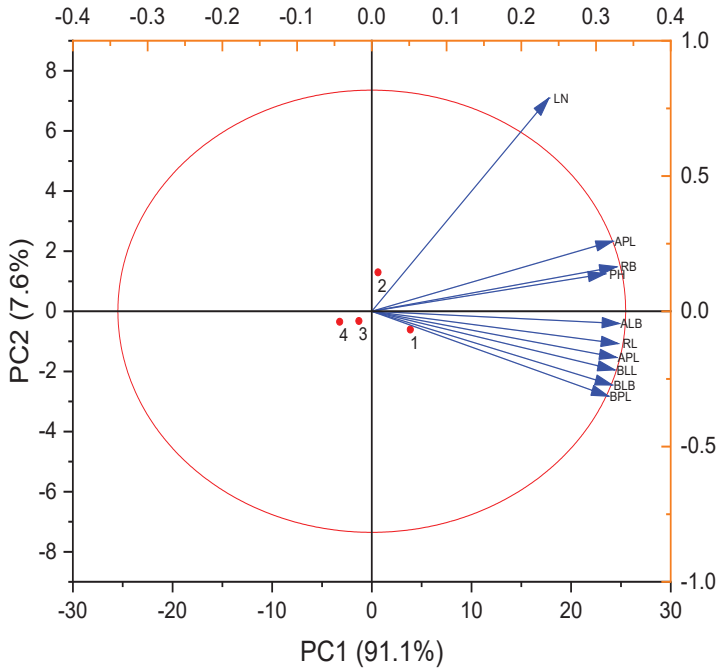

Fig. 7. Principal component analysis (PCA) of different morphological features of *P. cashmeriana* across different study sites. LN – Leaf number, APL – Apical petiole length, RB – Rhizome breadth, PH – Plant height, ALL – Apical leaf length, ALB – Apical leaf breadth, RL-Rhizome length, BLL – Basal leaf length, BLB – Basal leaf breadth, BPL – Basal petiole length; 1-KUBG ,2- Daksum,3- Hillar Naar, 4-. Jawahar tunnel

at different altitudes. The maximum variation can be seen in the overall resource budget and the total dry weight of above-ground plant parts in populations growing at KUBG (23.73±6.63 g) and Jawahar tunnel (12.94±7.67 g) representing minimum and maximum altitude respectively, while the total resource budget for sites Daksum and Hillar was 22.53±9.98 and 17.71±5.45 g. From Table 4 it is also clear that the partitioning of resources shows significant variation between different plant parts. Maximum resources are allocated towards rhizome followed by leaf and stem while minimum resources are allocated towards inflorescence. The maximum values were shown by the population growing at high altitude Jawahar tunnel (68.08±7.60 g) followed by populations present at Hillar and Daksum (48.09±8.59 & 48.10±13.38 g) respectively. While as the least value was shown by the population growing at KUBG (40.25±6.66 g).

3.3 Reproductive Success

Maximum % age reproductive effort per plant was shown by the population growing at high altitudes as compared to the one growing at lower altitudes, values range from 13.64 at Jawahar tunnel to 10.29 at KUBG respectively. From Table 3 it is clear that with the increase in altitude the no. of flowers per flower head as well as no. of flower heads per plant decreases. Maximum number of flowers per flower head and flower heads per plant were seen in KUBG (18.6±3.81 & 4±1.67) and minimum in Jawahar tunnel population (16.6±4.47 & 3.3±1.95) respectively. While as %age seed set also decreases with increase in altitude, highest being shown by population growing at KUBG (63.94 ±9.35) while as a minimum by population growing at J. tunnel (53.40 ±20.58).

4. DISCUSSION

Plants being sedentary possess remarkable phenotypic plasticity giving them an infinite potential for modification of physiological, morphological and reproductive attributes in response to changing environmental conditions. This is one of the dominantly studied plant developmental patterns throughout the globe [18, 19]. During the current study, it is observed that there is a discrete as well as directional vogue towards morphology, structural design, and characters associated with fitness among different surveyed populations of *P. cashmeriana* thriving at various altitudinal ranges. Noteworthy variability in phenotypic characters was documented and a general trend

Table 3. % age seed set (Mean ±SD) and floral dimensions across different study sites

Site	Jawahar tunnel	Hillar naar	Daksum	KUBG	F value
Av. Seeds Produced per flower head	44.4 ±18.45^{b*}	35.5 ±11.46^{a}	47 ±5.51^{a}	81.7 ±21.95^{a}	17.145
Av. Ovules borne	81.2 ±8.65^{b}	63 ±18.31^{a}	80 ±9.26^{a}	126.8 ±23.63^{a}	27.801
% age seed set	53.40 ±20.58^{a}	56.35 ±8.24^{a}	58.75 ±11.10^{a}	63.94 ±9.35^{a}	1.322
Flower no. per flower head	16.6±4.47^{a}	17.6±15.23^{a}	18.28±5.03^{a}	18.6±3.81^{a}	0.323
Flower head per plant	3.3±1.95^{a}	2.8±1.99^{a}	3.9±1.73^{a}	4±1.67^{a}	0.875
calyx length (cm)	1.72±0.20^{a}	1.61±0.18^{a}	1.5±0.22^{a}	1.62±0.13^{a}	2.351
calyx breadth (cm)	0.56±0.27^{a}	0.44±0.10^{a}	0.41±0.05^{a}	0.47±0.08^{a}	1.763
upper lip length (cm)	3.61±0.32^{b}	3.47±0.27^{b}	3.07±0.17^{b}	3.45±0.24^{a}	8.144
upper lip breadth (cm)	2.1±0.22^{b}	1.84±0.21^{b}	2.03±0.34^{b}	2.14±0.14^{a}	8.195
Lower lip length (cm)	3.17±0.36^{a}	3.09±0.19^{a}	2.65±0.32^{a}	3.22±0.20^{a}	1.962
Lower lip breadth (cm)	1.64±0.28^{b}	1.75±0.22^{b}	1.66±0.39^{b}	2.09±0.25^{a}	4.759
Androecium Length (cm)	2.7±0.23^{b}	2.74±0.20^{ab}	2.41±0.45^{ab}	2.87±0.22^{a}	4.116
Gynoecium length (cm)	4.16±0.15^{b}	3.75±0.41^{ab}	3.52±0.30^{a}	3.69±0.33^{a}	9.207

* Means labeled with the different small letters indicate that they significantly differ from each other among different populations

Table 4. Allocation of resources (Mean ±SD) towards vegetative and reproductive parts of *P. cashmeriana* at different sites

Traits	Jawahar tunnel (g)	Hillar naar (g)	Daksum (g)	KUBG (g)	F value
Rhizome	68.08±7.60^{a*}	48.09±8.59^{a}	48.10±13.38^{a}	40.25±6.66^{a}	1.904
Leaves	16.03±7.46^{c}	29.08±7.45^{c}	29.42±8.85^{b}	32.53±6.35^{a}	7.880
Stem	11.53±2.50^{c}	16.50±12.06^{bc}	16.51±7.72^{ab}	21.05±10.66^{a}	82.047
Inflorescence	4.35±2.02^{b}	6.31±2.97^{b}	5.95±1.99^{b}	6.15±2.34^{a}	12.520
Total resource budget per plant(gms)	12.94±7.67^{c}	17.71±5.45^{c}	22.53±9.98^{b}	23.73±6.63^{a}	22.756
Total reproductive effort per plant (%)	13.64^{a}	12.16^{a}	11.48^{a}	10.29^{a}	2.861

* Means labeled with the different small letters indicate that they significantly differ from each other among different populations

of linear decrease in plant traits with increasing altitude was more distinct and pronounced. A great deal of difference was found in plant height as it reduces with increasing altitude. Considerable phenotypic variability has also been found within and across the individuals of different populations of Ajuga bracteosa and Ajuga parviflora growing along different altitudes in Kashmir valley; the morphological characteristics showed both direct as well as a negative correlation with the altitude [20, 21]. Similar findings were revealed by other authors Hadi *et al*. (2022) [22], Korner *et al*. (2003) [23], Wills *et al*. (2002) [24] and Magray *et al*. (2021) [25] being one of the distinguished phenomena towards plant adaptation at increasing altitudes. The said outcome is due to a lower rate of growth as mitigation to stressful conditions and a shorter season for plant growth [26, 27]. The plants gain in such type of phenotypic character comes from evading strong winds blowing at higher altitudes besides enhancing photosynthetic efficiency via maintaining leaves in juxtaposition with warmer soil surface [28]. Besides, Lower mean annual temperature, high-intensity solar radiations, and high-altitude speedy winds limit plant growth and decrease leaf size with increasing altitude are the major reasons explaining this phenomenon. Apart from this decreased leaf size is also responsible for reduced solar radiation absorption which in turn helps in reducing the rate of transpiration and damages caused by high-intensity UV radiations.

The difference in flower number was also observed within the populations growing at different altitudes. The observed trend that plants growing at different altitudes follow suggests that those growing at lower altitudes are more dynamic, show maximum height and are capable of producing more flowers and leaves as compared to the ones growing at high altitudes. This conforms with the results of Johnson *et al*. (1968) [29], and Hickman *et al.* (1975) [30].

In addition to this, the association between the size of the plant and reproductive behavior (flower number); as already reported by Ohlson (1988), Aarssen and Taylor (1992), Kudo (1993) and Clauss and Aarssen (1994) [31-34]. To understand life history operative within a species, it is very important to understand the mechanism operative during resource allocation towards plant parts, within the overall resources allocated to the whole plant, plant size plays a pivotal role, which in turn reflects the potential of a plant to reproduce [35, 36]. *P. cashmeriana* the population under study shows a clear variation in portioning of biomass, as during the current study the population growing at high altitude has biomass allocated towards the stem, inflorescence, and leaves lower as compared to the below-ground part (rhizome). From the above results, it can be concluded that environmental selective forces result in deviation of reproductive strategies also reported by Shabir *et al*. (2013) [37]. Previous studies on some plant species have also proved that reproductive effort reduces considerably as plant size increases [38]. The reproductive effort of the plants growing at high altitudes was more than the plants growing at lower elevations. These findings confirm with Fabbro and Korner (2004) and Molina-Montenegro and Naya (2012), who reported a greater reproductive response with altitude

[39, 40]. Furthermore, the relationship of increased allocation to reproduction (number of flowers) with plant size is consistent with the observations of Aarssenand *et al*.(1992) [32], Clauss *et al*. (1994) [41].

5. CONCLUSION

The current studies reveal that *P. cashmeriana* prefers open rocky steep slopes. Plant species depict a wide range of variability in phenotypic traits along different altitudinal gradients of selected sites. While studying its phenotypic attributes, it was found that the population growing at lower elevations (KUBG) were taller and more vigorous than other studied populations growing at higher elevations. Contrary to plant height, the allocation of resource budget towards different parts showed a great variation, maximum resources were allocated towards rhizome followed by leaf, stem, and inflorescence. Further, reproductive success (fitness) was high in the populations growing at higher elevations as compared to the populations growing at lower elevations. Implying a clear prioritizing of reproduction over growth at high altitudes. This dynamics in both morphological and reproductive traits might be due to the reproductive effort, harsh environmental conditions and the presence of competitive habitat. We concluded that a heterogeneous environment is the primary cause of phenotypic variability in *P. cashmeriana*. Further studies related to the impact of other environmental aspects such as physicochemical properties of soil and climate would help us to understand if there are any other factors responsible for the phenotypic variability across different altitudes.

6. ACKNOWLEDGEMENTS

The first author is highly grateful to the Department of Botany, University of Kashmir for providing lab facilities during this research.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest

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Proceedings of the Pakistan Academy of Sciences: Part B
Life and Environmental Sciences 59(2): 49-64 (2022)
Copyright © Pakistan Academy of Sciences
ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(59-2)712

Pakistan Academy of Sciences

Research Article

Community Composition of Beetles (Insecta: Coleoptera) along Elevational Gradients in Phulchowki Hill, Lalitpur, Nepal

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Abstract: Beetles are recognized as important bio-indicators of the ecosystem that can be used to determine species diversity, genetic diversity, and ecosystem diversity. We investigated the species composition and diversity of beetles in four seasons along elevational gradients in Phulchowki hill from June 2018 to May 2019. Sampling was done using pitfall traps in five sites located at 1500 m, 1800 m, 2100 m, 2400 m, and 2700 m altitude respectively. Overall, we documented 43 morphospecies under 37 genera and 12 families from the study area. Scarabaeidae was the most dominant family whereas *Onthophagus sp*. 2 was the most abundant species in our study. The Shannon-Weiner diversity index, species richness and abundance were highest at 1500 m. Furthermore, diversity and species richness were highest in the spring, whereas peak beetle abundance was observed in summer. Principal component analysis (PCA) was performed to analyze the distribution patterns of the beetle families along the elevational gradients. PCA revealed a strong association of the Carabidae family with 1500 m, 1800 m, and 2100 m altitude whereas the Scarabaeidae family was mostly associated with human-influenced areas such as 1500 m and 2700 m altitude. The generalized linear model (GLM) revealed that temperature had a major impact on the overall beetle composition. Our study could set the standards for the research community to carry out conservation efforts on beetle diversity at different elevational ranges in the hill region.

Keywords: Abundance, Biodiversity conservation, Pitfall traps, Seasonal variations, Species richness

1. INTRODUCTION

Beetles (Insecta: Coleoptera) are dominant worldwide, constituting nearly a quarter of all known fauna [1]. They form great biodiversity in different habitats and play significant roles in the functioning of the ecosystem [2]. They occur in all major habitats, except for the Polar and marine habitats, and are economically important as agricultural and household pests or predators [3]. About 400,000 species of beetles have been identified worldwide [4] representing 211 families [5], with many more species yet to be discovered [6]. Sixty-three beetle families have been formally recorded from Nepal [7].

Beetles are recognized as important bio-indicators of the ecosystem that can be used to determine species diversity, genetic diversity, and ecosystem diversity [8]. A high diversity of beetles often indicates high diversity of other elements in an ecosystem [9]. Therefore, it is critical to understand global diversity and distribution patterns for assessing the status of overall biodiversity in the present crisis of mass extinction [10-12]. Furthermore, ground layer beetles show a wide range of distribution patterns in terms of geographical regions, climatic conditions, and vegetation patterns [13, 14] and are sensitive to environmental change [15-17]. Abundance, species richness, and composition of ground beetles are affected by the presence of tree canopy, leaf litter, and prey abundance in the forest [18] along with different habitat forms such as deciduous forest, which is important for maintaining rare species diversity [15].

Received: April 2022; Accepted: June 2022
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Environmental conditions change more quickly with altitude than with latitude, so mountain areas are thought to be an ideal location for investigating the relationship between biodiversity patterns and climatic components within spatial constraints [19-21]. Mountains provide steep environmental gradients [22] that contribute to high species diversity and draw the attention of conservationists [23]. The environmental variables, evolutionary factors and land use patterns collectively determine the biodiversity of montane ecosystems [24]. Moreover, beetle species composition also varies along elevational gradients [25, 26]. Species richness tends to decline with increasing elevation [27, 28] or peaks at mid-elevation [29-31]. The temperature has been identified as the primary predictor of species richness along with a few other factors such as relative humidity, soil nutrients, local habitat features, vegetation patterns, and available areas, all of which influence species diversity [32-34].

Phulchowki is the highest hill located in the mid-mountain region of Nepal. It offers a range of geographical slopes that support the inhabitation of a wide variety of flora and fauna. Despite the study area being recognized as a biodiversity hotspot, the beetle assemblages along elevational gradients had yet to be investigated. To understand the overall biodiversity of the mountain ecosystem, a shift in focus on the understudied beetles' community was necessary. Therefore, we carried out this study to: (i) assess species richness and abundance of beetles in the study area; (ii) compare the composition of beetle assemblages along elevational gradients of Phulchowki hill; (iii) determine the seasonal variation and (iv) investigate the relationships between the beetle community and environmental variables (temperature and humidity). The main purpose of this study was to explore the community structure of beetles associated with ecological and environmental components of the mid-hill region. Moreover, this research will help to conduct further studies and implement conservational strategies for the Coleopteran diversity in Nepal.

2. MATERIALS AND METHODS

2.1 Study Area

Phulchowki hill (Latitude: 27°35'00" N and Longitude: 85°24'00" E) is situated in the Lalitpur district of Nepal. Its elevation ranges from 1500 m to 2762 m. Forest is covered by shrubs, herbs and trees and therefore represents a diverse floral assemblage. The Phulchowki hill is characterized by three distinct evergreen broad-leaved forest types: mixed *Schima-Castanopsis* forest at the base (1500 m - 1800 m), Oak- Laurel forest (1800 m - 2400 m) and evergreen oak forest (above 2000 m) [35]. Phulchowki is 4281 ha in size, one-third of which is managed as a community forest (1368 ha), and the rest (mainly on and around the summit) is a national forest [36]. The elevational gradients ranging from 1500 m to 2700 m were chosen for the study (Fig. 1).

2.2 Study Design

The study area was divided into five sampling sites maintained at 1500 m (27°35'18" N, 85°22'47" E), 1800 m (27°34'53" N, 85°22'57" E), 2100 m (27°34'44" N, 85°23'30" E), 2400 m (27°34'38" N, 85°23'54" E) and 2700 m (27°34'16" N, 85°24'13" E). A survey was conducted from June 2018 to May 2019 that covered four different seasons (summer, autumn, winter, and spring). The pitfall trap method was used to collect beetles at each site [37]. Ten pitfall traps were set up in each sampling site within a 300 sq. m area. Each trap was spaced three meters apart from the others and five meters away from the forest fragment border. The traps were then filled with one-fourth of water and a few drops of ethylene glycol as preservatives [38]. All of the traps were set up from 11 am to 4 pm on the same day and then left for one week. These traps were kept in the same place during all seasons. The specimens from each trap were separated from debris and unwanted particles and preserved at 70 % ethyl alcohol. Then labels with site and sample numbers were marked on the vials. Further lab works were conducted at the Department of Zoology, Amrit Campus.

2.3 Identification and Categorization of Specimens

The specimens were sorted into their respective families and assigned to morphospecies based on their morphological features. Different taxonomic keys were used for the identification of specimens up to the family and morphospecies levels [39-42]. We further compared these specimens with labeled beetle specimens available at Natural History

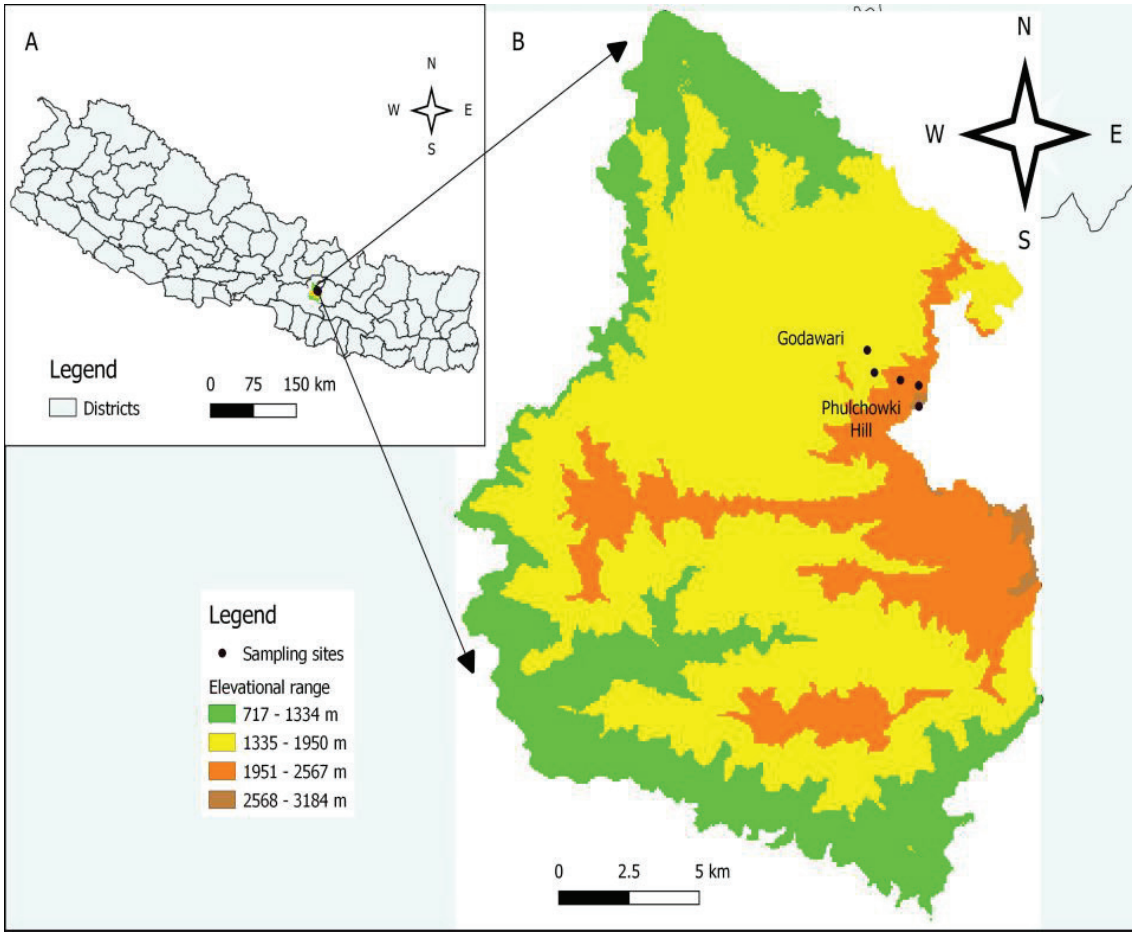

Fig. 1. Map of the study area. A) Map of Nepal showing Lalitpur district; B) Elevation map of Lalitpur district showing sampling sites located at Phulchowki hill

Museum, Swoyambhu, Kathmandu to identify them.

2.4 Data Analysis

Shannon-Weiner (H′) and Pielou´s evenness (J) were estimated for calculating the species diversity of the beetle in study area [43, 44]. Bray Curtis's analysis for hierarchical clustering using the single linking method was used to analyze the similarities among the beetle assemblages. Principal component analysis (PCA) was done to analyze the distribution patterns of beetles among the altitudinal gradient. The data were normalized before analysis. Furthermore, we evaluated the averages of environmental variables i.e. temperature and humidity from our recorded data. These data were measured by ourselves during the sampling period in the study field using a digital thermo-hygrometer (HTC-2). The relationship of temperature and humidity with the species richness and abundance of beetles was tested by generalized linear modeling (GLM) [45]. Data were analyzed using the vegan package [46] in R software version 3.6.1 [47].

3. RESULTS

3.1 Species Richness and Abundance of Beetles

Overall 237 beetle specimens were collected during the survey representing 43 morphospecies under 37 genera and 12 families (Scarabaeidae, Carabidae, Coccinellidae, Chrysomelidae, Silphidae, Staphylinidae, Curculionidae, Megalopodidae, Prionoceridae, Cantharidae, Cleridae, and Tenebrionidae) (Table 1). The Carabidae family had the most species (11) followed by Chrysomelidae (10), Scarabaeidae

Table 1. Beetle species recorded from different elevations in Phulchowki hill

S.No.	Family	Morphospecies	Presence (+) or absence (-) of morphospecies at different elevations					Abundance (%)
			1500 m	1800 m	2100 m	2400 m	2700 m	
1	Cantharidae Imhoff, 1856	*Athemus trimaculatus* Hope, 1831	+	-	-	-	-	1.27
2	Carabidae Latreille, 1802	*Abax* sp.	-	-	+	-	-	1.27
3		*Agonum* sp. 1	+	+	-	-	-	1.27
4		*Agonum* sp. 2	-	+	-	-	-	0.84
5		*Carabus* sp.	-	-	-	-	+	0.84
6		*Cicindela* sp.	-	+	-	-	-	0.42
7		*Craspedophorus* sp.	-	+	-	-	-	0.42
8		*Harpalus* sp.	+	+	+	-	-	2.11
9		*Nebria* sp.	+	+	-	-	-	8.86
10		*Platynus* sp.	-	+	-	-	-	0.42
11		*Pterostichus* sp. 1	-	-	+	-	-	0.84
12		*Pterostichus* sp. 2	-	-	+	+	+	2.11
13	Chrysomelidae Latreille, 1802	*Aplosonyx* sp.	-	-	-	+	-	0.42
14		*Callosobruchus chinensis* Linnaeus, 1758	-	-	-	-	+	1.27
15		*Chaetocnema* sp.	+	+	+	-	-	5.91
16		*Chrysomela* sp. 1	+	-	-	-	-	1.27

S.No.	Family	Morphospecies	Presence (+) or absence (-) of morphospecies at different elevations					Abundance (%)
			1500 m	1800 m	2100 m	2400 m	2700 m	
17		*Chrysomela* sp. 2	-	-	-	+	+	1.27
18		*Cryptocephalus* sp.	-	-	-	+	-	1.27
19		*Gonioctena* sp.	-	-	-	+	-	0.42
20		*Hoplasoma* sp.	+	-	-	-	-	0.42
21		*Hyphasis* sp.	-	+	-	-	-	0.42
22		*Lema* sp.	+	-	-	-	-	0.42
23	Cleridae Latreille, 1802	*Onychotillus* sp.	+	-	-	-	-	0.84
24	Coccinellidae Latreille, 1802	*Coccinella* sp.	+	+	+	+	+	13.08
25	Curculionidae Latreille, 1802	*Otiorhynchus* sp.	+	+	-	+	-	6.33
26		*Phyllobius* sp.	-	+	-	-	-	0.42
27		*Trypodendron* sp.	-	+	-	-	-	0.84
28	Megalopodidae Latreille, 1802	*Zeugophora* sp.	-	-	-	+	-	0.42
29	Prionoceridae Latreille, 1802	*Idgia melanura* Kollar and Redtenbacher, 1844	+	-	-	-	-	0.84
30	Scarabaeidae Latreille, 1802	*Anomala* sp.	-	+	-	-	-	0.42
31		*Cetonia* sp.1	+	-	-	-	+	1.27

S.No.	Family	Morphospecies	Presence (+) or absence (-) of morphospecies at different elevations					Abundance (%)
			1500 m	1800 m	2100 m	2400 m	2700 m	
32		*Cetonia* sp. 2	+	-	-	-	-	0.84
33		*Onthophagus gagates* Hope, 1831	+	-	-	-	+	1.69
34		*Onthophagus* sp. 2	+	+	+	+	+	30.80
35		*Phyllophaga* sp. 1	+	-	-	+	-	0.84
36		*Phyllophaga* sp. 2	-	-	-	-	+	0.42
37	Silphidae Latreille, 1806	*Nicrophorus* sp.	-	+	-	-	-	1.69
38	Staphylinidae Latreille, 1802	*Creophilus* sp.	-	-	-	+	-	0.84
39		*Ocypus* sp.	-	-	+	-	-	0.42
40		*Oxytelus* sp.	+	-	-	-	-	0.84
41		*Stenus* sp.	+	+	-	-	-	1.27
42		*Xantholinus* sp.	-	-	-	+	-	1.27
43	Tenebrionidae Latreille, 1802	*Gonocephalum* sp.	-	-	-	+	+	0.84

(Seven), Staphylinidae (Five), and Curculionidae (Three). The families Coccinellidae, Tenebrionidae, Cleridae, Prionoceridae, Megalopodidae, Cantharidae and Silphidae were represented by single species. A considerable difference in beetle abundance was observed during the study period. We recorded the Scarabaeidae family (36.28 %) as the most dominant family. Overall, *Onthophagus* sp. 2 (30.80 %), *Coccinella* sp. (13.08 %), *Nebria* sp. (8.86 %), *Otiorhynchus* sp. (6.33 %), and *Chaetocnema* sp. (5.91 %) were the most abundant species. *Onthophagus* sp. 2 and *Coccinella sp*. were recorded from all sites.

3.2 Composition of Beetles along the Elevational Gradients

The highest number of species (19) were collected at 1500 m elevation while the least number of species (Eight) were recorded at 2100 m elevation. On the other hand, the abundance of beetle was highest (88) at 1500 m elevation whereas the lowest abundance (20) was observed at 2700 m elevation (Fig. 2).

The Shannon-Weiner diversity index (H′) revealed the highest beetle diversity (H′=2.42) at 1500 m elevation whereas the lowest diversity (H′=1.72) at 2100 m and 2700 m elevations. The evenness index was recorded at maximum (J=0.92) at 2400 m elevation (Table 2).

PCA analysis for Coleoptera assemblages along the elevational gradients predicted the association of beetle families with particular elevations investigated. The first two principal components of the PCA biplot explained 87.9 % and 7.8 % of the total variation (Table 3). There was a strong correlation of species found at 1500 m and 1800 m elevations. A high correlation of 2100 m elevation was observed with 1500 m and 1800 m elevations. Similarly, 2700 m elevation also had a high correlation with 1500 m and 1800 m elevations. However, the beetle's composition of 2400 m elevation was differentiated from any of other elevations (1500 m, 1800 m, 2100 m, and 2700 m). There was a negative correlation of 2400 m and 2700 m elevations with principal component 1 (PC1) in which 2400 m showed a highly negative correlation with PC1. On the other hand, a positive correlation of 1500 m, 1800 m, and 2100 m was viewed with principal component 2 (PC2) (Fig. 3). Species of the Carabidae family were largely associated with 1500 m, 1800 m, and 2100 m elevations. Furthermore, the composition of the Scarabaeidae family was related to 1500 m and 2700 m elevations.

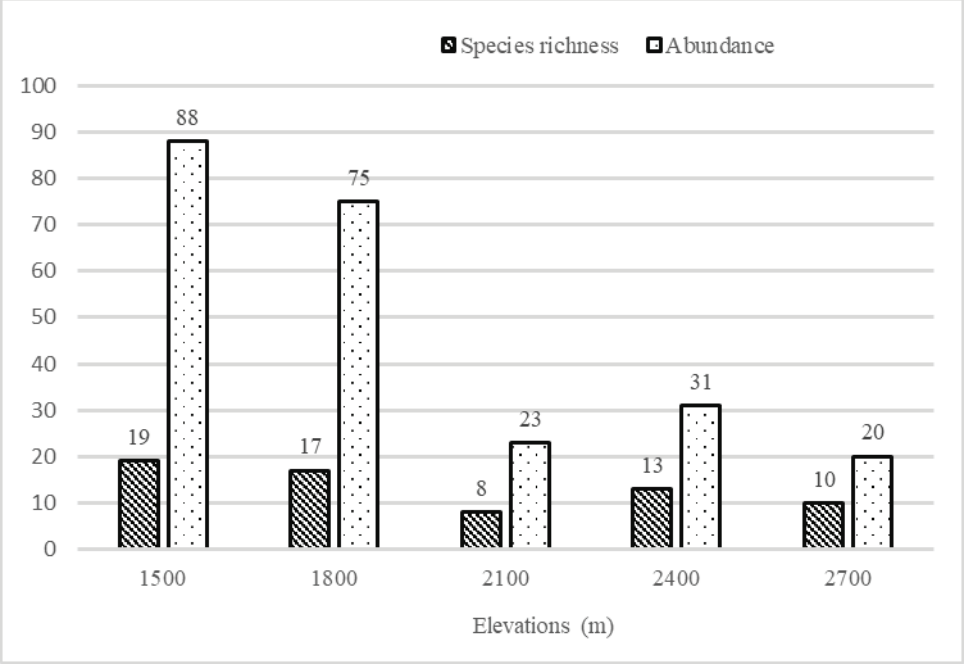

Fig. 2. Species richness and abundance of beetles along elevational gradients

Table 2. Main attributes of beetle assemblages in Phulchowki hill

Attributes	Elevations					Seasons			
	1500	1800	2100	2400	2700	Summer	Autumn	Winter	Spring
Number of families	9	7	4	9	5	7	7	3	10
Number of morphospecies	19	17	8	13	10	10	16	4	23
Shannon-Wiener index (H′)	2.42	2.18	1.72	2.36	1.72	1.25	2.38	1.03	2.50
Pielou index (J)	0.82	0.77	0.83	0.92	0.72	0.54	0.86	0.74	0.79

Table 3. Summary of Principal Component Analysis

Attributes	PC1	PC2	PC3	PC4	PC5
S.D.	2.0967	0.6249	0.34079	0.28529	0.12515
Proportion of Variance	0.8793	0.0781	0.02323	0.01628	0.00313
Cumulative proportion	0.8793	0.9574	0.98059	0.99687	1

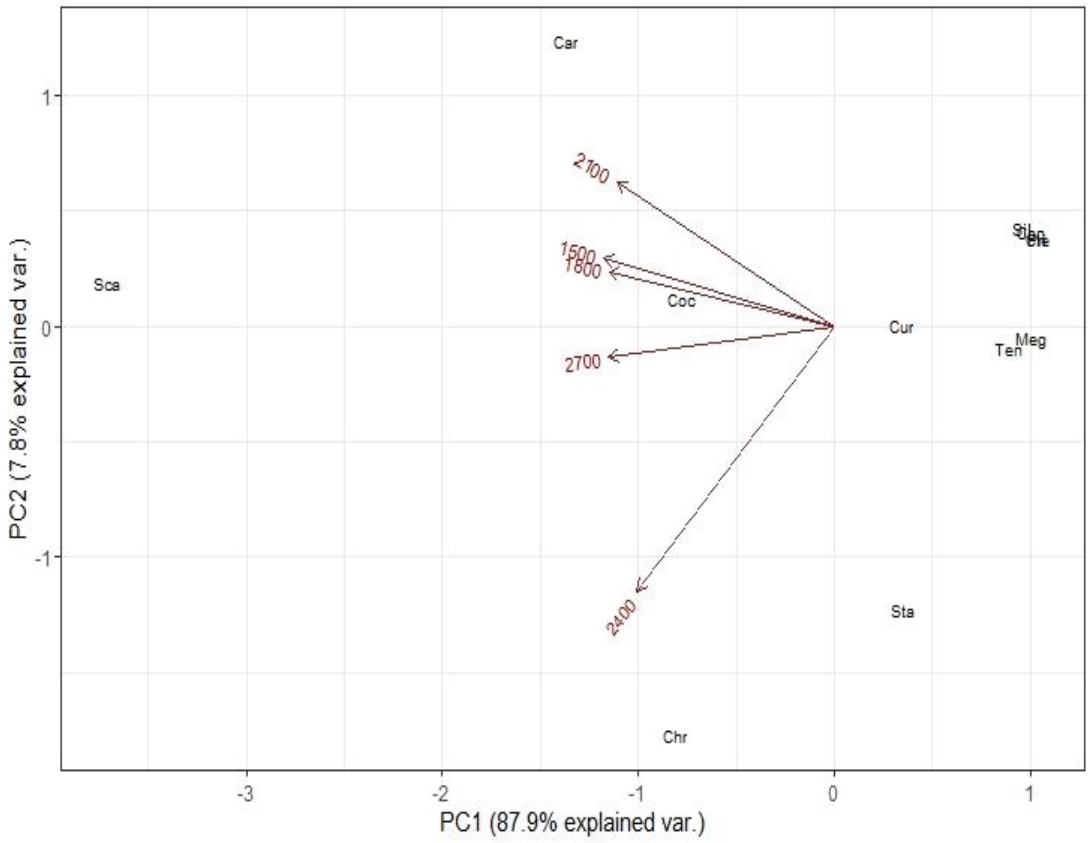

Fig. 3. Biplot of PCA for assemblages of Coleoptera families in five elevations

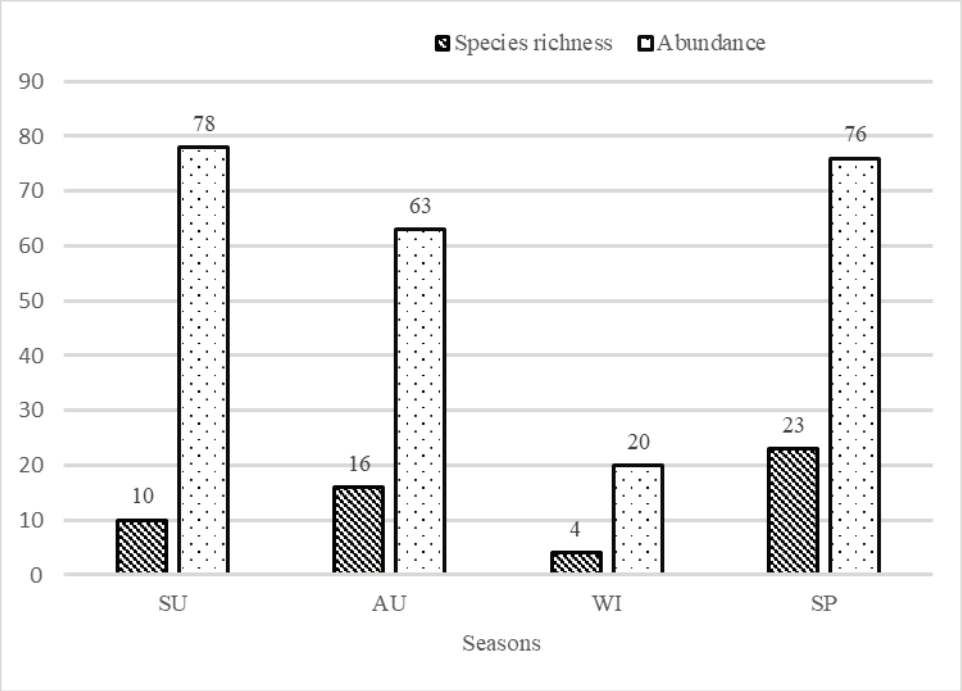

Fig. 4. Species richness and abundance of beetles in four seasons. Seasons symbols:- SU: Summer, AU: Autumn, WI: Winter, SP: Spring

3.3 Seasonal Variation of Coleoptera

Species richness was found to be the highest (23) in the spring season while the lowest number of species (four) were captured during the winter season. However, coleopteran abundance was highest (78) in the rainy summer season and least (20) in the dry winter season (Fig. 4). Furthermore, the Shannon-Weiner diversity index (H′) revealed that the beetle diversity peaked during the spring season (H′=2.5) while bottomed during the winter season (H′=1.03). In contrast to this, the Pielou′s evenness was more or less similar in winter and spring but was found to be maximum in autumn (J=0.86) (Table 2).

The hierarchical clustering dendrogram by cluster analysis depicted similarities in beetle composition between four different seasons such as summer, autumn, winter, and spring. A similar beetle composition was observed between the autumn, summer, and spring seasons. However, the beetle composition in the winter season was least similar to other seasons during the study (Fig. 5).

3.4 Relationships between Beetle Community and Environmental Variables

Environmental factors such as temperature and humidity were used as significant predictor variables (independent variable) whereas beetle abundance and species richness were used as the response variables in the General linear modeling (GLM). According to the results of analysis done using General linear modeling (GLM) with Poisson regression, there was an association between temperature and humidity with the beetle community throughout our study period in Phulchowki hill. It revealed that beetle abundance was significantly influenced by both temperature ($z = 8.211$, $p < 2e\text{-}16$) and humidity ($z = 3.827$, $p = 0.00013$) (Table 4). However, the species richness of beetle was significant with only temperature ($z = 2.263$, $p = 0.0236$). There was no significant impact on the species richness of beetles by humidity ($z = 1.707$, $p = 0.0879$) (Table 5). Overall, this finding indicated that the temperature [abundance ($p < 2e\text{-}16$) and species richness ($p = 0.0236$)] was the best predictor variable than

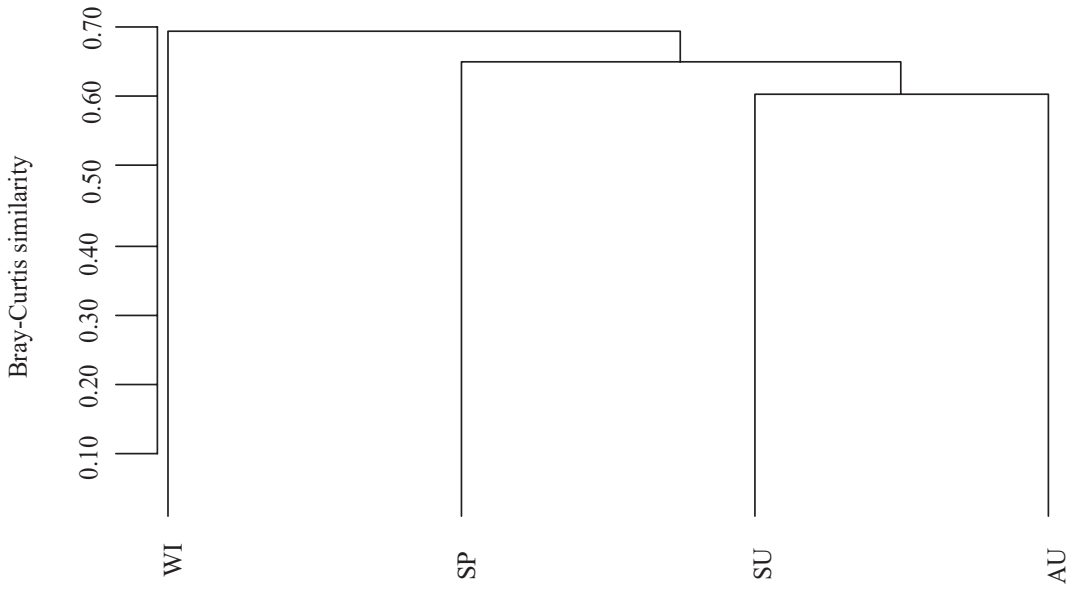

Fig 5. Cluster Dendrogram by Bray Curtis Analysis (single linkage) for beetle assemblages of seasons studied. Season symbols: SU-Summer, AU-Autumn, WI-Winter, SP-Spring

Table 4. Relation of beetle abundance with environmental factors (Generalized linear modeling with Poisson regression using log link function)

Factors	Estimate	Std. Error	z value	p-value
Temperature	0.28590	0.03482	8.211	< 2e-16
Humidity	0.05644	0.01475	3.827	0.00013

Table 5. Relation of species richness with environmental factors (Generalized linear modeling with Poisson regression using log link function)

Factors	Estimate	Std. Error	z value	p-value
Temperature	0.13008	0.05748	2.263	0.0236
Humidity	0.04381	0.02567	1.707	0.0879

humidity [abundance ($p = 0.00013$) and species richness ($p = 0.0879$)] as it greatly affected presence of beetle species along with their abundance and hereby shaped the patterns of beetle community in Phulchowki hill.

4. DISCUSSION

4.1 Species Richness, Abundance and Diversity of Coleoptera along Elevational Gradients

Family Scarabaeidae was most abundant in our study. Satheesha *et al*. [48] also reported Scarabaeidae to be the predominant family in their research from different habitat sites of Davangere University Campus, Karnataka, India. The occurrence of dung-producing mammals in forests could be the reason for the higher abundance of dung beetles [49]. Abundance and species richness, as well as beetle diversity, was observed maximum at the lowest elevation. Musthafa *et al.* [50] recorded the peak diversity at lower elevations. Species richness is considered an indispensable factor to estimate the biodiversity of an ecosystem. A greater number of species were associated with lower elevations than upper elevations. A similar species diversity

pattern along increasing elevations at Genting Highland, Malaysia has been documented by Musthafa and Abdullah [8]. Likewise, Gebert *et al* [51] observed abundance to reach a peak at around 1500 m when abundances of beetle species were investigated from 870 m to 4500 m elevation. In addition, variables such as habitat, food availability, vegetation structure and leaf litter are responsible for the diversity and abundance of terrestrial insects [52-54]. Therefore, the availability of more food resources and mixed vegetation at lower elevations could be the reason for the maximum abundance of beetles.

The Carabidae family was predominantly associated with lower elevations such as 1500 m, 1800 m, and 2100 m. These elevations provided different geographical gradients and microhabitats such as caves, endogean, ant nests, termite tubes, leaf litter, tree bark, under logs, rocks, edge of small water bodies, small grassland areas, etc. Ground beetles displayed strong mobility between various ecosystems [55, 56] and their population and species diversity were positively correlated with habitat diversity [57-60]. Most species of Scarabaeidae were recorded from 1500 m and 2700 m elevations. Anthropogenic activities such as manufacturing industries, educational institutes, Godawari buspark, and construction projects around 1500 m elevation and the presence of military camps, temples and tourism-related activities at the top of the hill could be responsible for the existence of many dung beetle species. Musthafa and Abdullah [61] recorded maximum dung beetles in the same way from the high human-influenced area in a recent study. Dung beetle populations are used to determine the land use pattern and effects on biodiversity by human interactions [62].

4.2 Seasonal Variation of Coleoptera

Grouping of pairs by cluster analysis of seasons showed identical beetle assemblages in the summer, autumn, and spring season. However, beetle composition in winter was least similar to other seasons. The presence of favorable vegetation like tree canopies, suitable temperature, and excessive foraging materials contributed to the highest collection of beetle species during the spring season. The outcome of our study was closely related to Silva *et al*. [63], which also recorded the highest number of beetle species during the spring season in the forest fragments of Brazil. Furthermore, our study demonstrated the highest abundance of beetle in the summer season. We observed this result due to heavy rainfall and warm temperature during the summer season. The least abundance was reported in the winter season. Moreover, the dung beetle's composition was affected by the seasons as well and thus, does not occur uniformly throughout the year. The result was firmly associated with the finding of Jain and Mittal [49], which documented the highest abundance of dung beetles during wet summer in the forests of Haryana (India). Similarly, Wardhaugh *et al*. [64] reported the highest abundance of beetle during the wet summer season and the lowest during the dry winter season. Arya *et al*. [65] also recorded the most number of beetle individuals in the rainy season along the altitudinal gradient of Binsar Wildlife Sanctuary, Almora, Uttarakhand, India.

4.3 Relationships between Beetles Community and Environmental Variables

General linear model (GLM) results indicated that both beetle abundance and species richness were affected by temperature. This was further concluded by Moraes *et al*. [53] in their study of Carabid beetles in humid forests of southern Brazil. Contrary to this, the humidity only had a significant impact on the abundance of the beetle. However, no major effect was obtained on species richness due to humidity. The number of species presence greatly influenced the overall beetle diversity. The maximum species richness and beetle diversity were observed in the spring season when the temperature was highest. During cold winter, number of species and diversity were lowest. A study conducted by Nunes *et al*. [22] on dung beetles revealed the decline of dung beetle richness on decreasing temperature. As a whole, the temperature was the best predictor variable than humidity as it often determined the beetle community in Phulchowki hill. Wardhaugh *et al*. [64] also highlighted temperature as a major environmental variable to explain the total abundance and species diversity patterns. Likewise, Oliveira *et al*. [66] addressed the strong and significant correlations between Coleoptera abundance and temperature. Nevertheless, temperature and humidity were accountable for the beetle abundance and their activities [67]. This result was in accordance with

the studies of seasonal variation of arthropods in the tropical region [68, 69]. Furthermore, Louzade and Lopes [70] reported the highest number of Scarabaeinae species during the hot summer season in the forests of Brazil. The study on the diversity and seasonal abundance of Scarabaeoid dung beetle in Central New Jersey showed a significant effect of temperature on the abundance of dung beetle captured each month in farm sites [71]. Similarly, Hernández and Vaz-de-Mello [72] found a positive correlation and a linear relationship between the species richness of beetles and the mean monthly temperature in the forest ($p < 0.01$).

There were a few potential limitations in our study. Some of them are mentioned as follows: (i) taxonomy problem for species-level identification of beetle specimens; (ii) difficulty in field visits and data collection due to heavy rainfall during the summer season; (iii) lack of prior research on beetles community in hills region of Nepal and (iv) lack of authoritative climatic data from concerning department.

5. CONCLUSION

This study from Phulchowki hill recorded a total of 237 beetle individuals representing 43 morphospecies belonging to 37 genera and 12 families. Beetle assemblages were more diverse at lower altitudes. The highly abundant Scarabaeidae in human-influenced areas such as 1500 m and 2700 m indicated the growing anthropogenic pressure in the hill region. The Carabidae was the most diverse family which were largely associated with 1500 m, 1800 m, and 2100 m altitudes. Overall, there was a high correlation of species composition between 1500 m and 1800 m elevations. Seasonally, the diversity peaked during the warm spring season and down during the cold winter season. Furthermore, the composition of beetles in the autumn and summer seasons was similar. Our results indicated that temperature had a strong influence on the composition of Coleoptera assemblage. This study in Phulchowki hill can be used to evaluate the elevational beetle diversity pattern in the mountain region in Nepal. It could further set the standards for the research community to carry out conservation efforts.

6. RECOMMENDATIONS

More information on beetle composition might be crucial as their species distribution patterns can be applied as a bioindicator. It can effectively determine the human impact on the mountain ecosystem and thereby help us to execute biodiversity conservation strategies in Nepal. Therefore, extensive research in a wide range of elevations in the mountain region is necessary for a better understanding of overall biodiversity.

7. ACKNOWLEDGEMENTS

We would like to thank the Department of Forests and Soil Conservation (Ministry of Forests and Environment), Kathmandu for the permission to conduct this research in Phulchowki hill. Furthermore, we are very grateful to all staff members of the Department of Zoology, Amrit Campus for their cooperation and support during this work. We express our deep gratitude to Mr. Shambhu Adhikari, Amrit Campus for his support and suggestion during the research. Credits also go to our friends Rachana Pandey, Manisa Singh and Tulsi Raj Adhikari for their involvement in the field data collection. We extend our gratitude to Prof Dr. Dharma Raj Dangol and Dr. Ishan Gautam, Natural History Museum, Swoyambhu for providing access to the beetle specimens housed at Natural History Museum, Tribhuvan University.

8. CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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Proceedings of the Pakistan Academy of Sciences: Part B
Life and Environmental Sciences 59(2): 65-70 (2022)
Copyright © Pakistan Academy of Sciences
ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(59-2)695

Pakistan Academy of Sciences

Research Article

Genetic Analysis of Restriction Fragment Length Polymorphism of TLL1 Gene (rs17047200) in Patients of Hepatocellular Carcinoma

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Abstract: To find out the association of polymorphism of the TLL1 gene in hepatocellular carcinoma. A cross-sectional study was conducted from January 2020 to September 2020. Subjects were enrolled from Mayo Hospital, Lahore, Jinnah Hospital Lahore and the Liver Transplant Unit of Sheikh Zayed Hospital, Lahore. A total of 200 individuals were registered and segregated into the Control group (n=100) and hepatocellular carcinoma (HCC) group (n=100). DNA was extracted from obtained blood samples and Restriction Fragments Length Polymorphism (RFLP) was carried out at the laboratories of LCWU by using specific primers and restriction endonuclease enzymes. The data were analyzed statistically. The high proportion of smoking, hepatitis B, hepatitis C, cirrhosis and Body Mass Index (BMI) were established risk factors in the HCC group. Subjects with hepatocellular carcinoma had low socioeconomic status. Heterozygous bands in the HCC group were observed after RFLP. TLL1 genotype was AA (72 %) and AT/TT (28 %). The patient's clinical aspects were similar across TLL1 genotypes. It was concluded that RFLP on the exon region by using their specific enzymes HpyCH4III showed heterozygous bands in the HCC group that indicated a mutation in the TLL1 gene though this mutation does have a significant association with HCC.

Keywords: Liver Cancer, Polymorphism, Hepatocellular Carcinoma, PCR, TLL1 gene

1. INTRODUCTION

Globally, the prevalence of liver cancer is increasing which is also acknowledged as primary hepatic cancer or primary hepatic malignancy. Hepatocellular carcinoma (HCC) includes about 80 percent of intrahepatic cholangiocarcinoma. In addition, HCC is linked to abdominal mass and pain, anemia, itching, fever, back pain, weight loss, and jaundice [1]. Moreover, it is more common in men as compared to women [1]. In the past few years, the landscape of hepatocellular carcinoma (HCC) has been rapidly growing and also accounts for approximately 75 percent of all primary liver malignancies [2]. Cancer-related death was caused due to HCC. The incidence of Hepatocellular carcinoma (HCC) shows substantial global variation which is dependent on the differences in HCC risk factors including exposure to co-carcinogens as well as viral hepatitis. The highest incidence of Hepatocellular carcinoma had shown in sub-Saharan Africa and Southeast Asia [1, 3, 4]. the incidence and cancer mortality is increasing in the developing world. Similarly, Pakistan faces disturbing limitations in cancer care that have an adverse impact on patient outcomes [5].

Numerous risk factors include smoking, hepatitis B virus, cirrhosis, metabolic disease, genetically liver disorders, diabetes, lack of antioxidant vitamins and selenium, non-alcoholic fatty liver disease, long-term excessive drinking, intake of aflatoxin, hepatitis C virus, in women with a long-term oral contraceptive, chronic and inorganic lead poisoning, and iron-overload are the prime peril for the hepatic tumor. Inclusion of contaminants and exposure to risk factors proves to be the reason for the appalling liver cancer. Hereditary vulnerability of hepatic tumors becomes the main focus of research [6, 7].

Received: December 2021; Accepted: June 2022
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Mammalian tolloid-like 1 is zinc-dependent matrix metalloproteases which are also known as TLLI and belong to a subfamily known as the bone morphogenetic protein 1 as BMP1 as well tolloid-like proteinases (BTPs). BMP1 is the first discovered tolloid of the family in mammals and is also known as procollagen C-endopeptidase, which involves the formation of the extracellular matrix. In humans, Tolloid-like protein 1 is encoded on the TLL1 gene located on chromosome 4 [8]. In hepatocarcinogenesis after hepatitis c virus elimination or liver fibrosis in a patient with nonalcoholic fatty liver disease, the single nucleotide polymorphism in Tolloid-like 1 and expression of TLL1 were closely related [9].

In our study, we aimed to analyze the genetic factors in the Pakistani population and the role of polymorphism of TLL-1 (rs17047200) in association with liver cancer. To the best of our knowledge, this is the first study for genetic analysis of RFLP of the TLL1 gene (rs 17047200) in Pakistani subjects of HCC.

2. METHODOLOGY

The study was conducted in different hospitals including Mayo Hospital, Jinnah Hospital and Sheik Zayed Hospital, Lahore in Lahore from January 2020 to September 2020. Patients who have Hepatocellular Carcinoma (HCC) were recognized after they were clinically examined by the doctors. Healthy subjects were also enrolled as a control group and both groups were ethnically matched. The Ethical Committee of LCWU and hospitals approved the study. Information of the enrolled subjects regarding age, sex, family history, weight, height, body mass index (BMI) the antecedent of other cancers, carcinogenic treatments, contraindication related to surgery, pregnancy-related issues, embryo-procure tumor, agile liver ailment, and the other factors were collected through a self-designed questionnaire.

2.1 Criteria Used

For study subjects the inclusion and exclusion criteria were as follow:

2.1.1 Inclusion Criteria

Subjects who were suffering from liver cancer like Hepatocellular Carcinoma, as well as fibrosis together with liver cirrhosis, were taken into consideration in this study, and subjects without any disease were referred to as a control group.

2.1.2 Exclusion Criteria

Subjects who have any other disease such as cardiovascular disease or kidney disease were excluded from this study as well as from the control group.

Before the collection of blood samples, the informed consent form was taken from each subject. The collected blood samples were stored at -20 ºC and DNA was separated by following the procedure of Gimberg *et al.* (1989) [10]. The polymerase chain reaction-restriction length polymorphism (PCR-RFLP) technique was adopted to analyze the TLL1 gene polymorphisms using specific primers as shown in Table 1. Gel electrophoresis was carried out to see the products of enzyme digestion.

Table 1. The primers of the TLLI gene

S. No.	Gene	Primer	GC Content	Product Size
1	TLL1 (R)	5/ CCA TAG GAA GCA ATG CTG AAC 3/	47.62	492bp
2	TLL1 (F)	5/ CTG TTG ACC TAA GAC GTA ATG G-3/	45.45	

2.2 Statistical Analysis

All statistical analysis was carried out using Microsoft Excel and Statistical data for Social Sciences (SPSS, version 19.0), and the t-test was used to compare the means of two groups.

3. RESULTS

The Clinical Attributes of the study groups were presented in Table 2.

Molecular analysis of the TTL1 gene was conducted using Polymerase Chain Reaction (PCR) restriction fragments length polymorphism (RFLP) in both the control and HCC groups. After DNA amplification of the exon VII region by using its

respective primers, we got the PCR products of 492bp when compared with the ladder as shown in figure 1 & figure 2.

Restriction Fragments Length Polymorphism (RFLP) was performed to analyze if any polymorphism of the TTL-1 gene was present. RFLP was performed on the exon region by using their specific enzyme HpyCH4III. The Heterozygous bands in the HCC group obtained through RFLP indicated mutation. The homozygous bands in the control batch had appeared. TLL1 genotype was AA (72 %) and AT/TT (28 %). The patient's clinical aspects were similar across TLL1 genotypes. In the HCC group, the heterozygous bands appeared which shows the association of the TTL-1 gene with hepatocellular carcinoma.

Table 2. Demographic data of the studied population

Variables	Control group	HCC group
Age (years)	29.8 ± 5.11	38.3 ± 7.14**
Weight (kg)	59.5 ± 9.79	61.3 ± 6.61**
Height (cm)	5.26± 0.54	5.20 ± 0.58
BMI (kg/m^2)	24.4 ± 5.79	26.14 ± 6.61*

*Significant variation between groups (p≤0.05).
**Highly notable difference among the groups (p≤0.01)

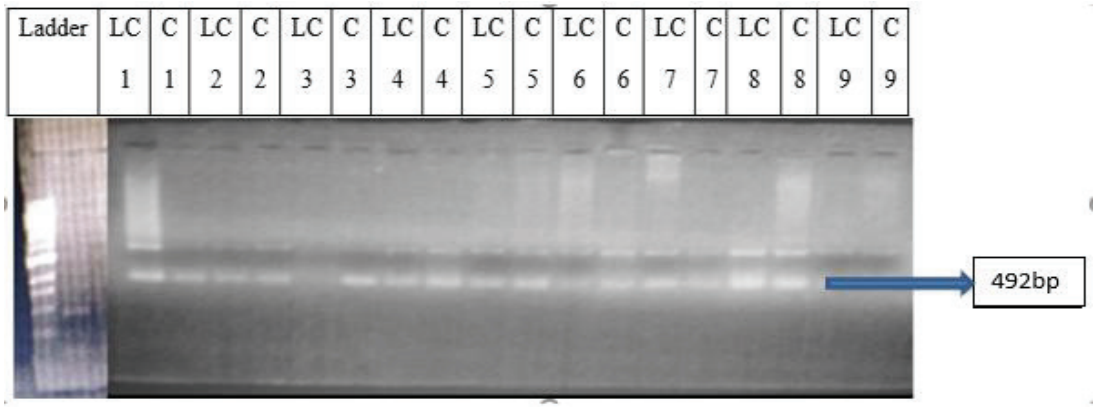

Fig. 1. PCR product obtained by using specific primers

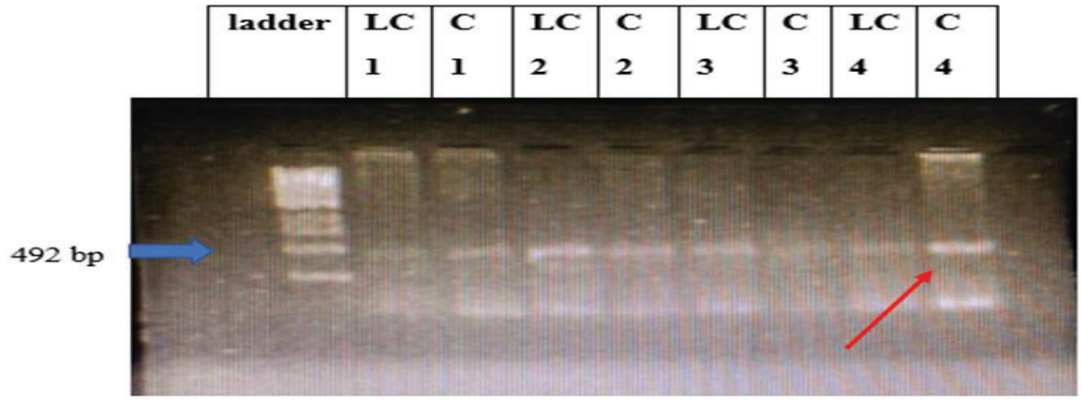

Fig. 2. RFLP performed for the digestion of amplified PCR products.

4. DISCUSSION

HCC is not only the sixth most widespread category of cancer in the world but also the third uncouth reason for cancer deaths [11, 12]. The prevalence of hepatocellular carcinoma was high in males (80 %) than the females (20 %) ($p \leq 0.01$). The current study depicts that the prevalence of hepatocellular carcinoma is high in males as compared to females. A similar result has been reported by the American cancer society, (2016) that the probability of liver cancer significantly soars high in males as compared to females. Obesity is a major complication. A report by the world health organization report describes that obesity all over the world has been two-fold since 1980. Our study depicts that in the hepatocellular carcinoma group 65 % of individuals were found overweight. It has been found that there is more prevalence of overweight in the HCC group compared with the control group.

It has been assessed in another study that the risk of developing liver cancer is increased due to being obese which could be resulted in cirrhosis and fatty liver disease [1].

Smoking is one of the leading sources of hepatocellular carcinoma and is also the major cause of many diseases. In the present study, the percentage of smokers was high in the HCC group in comparison with the control group. In the present study, in the hepatoma group, the pervasiveness of current and former smokers was 25 % and 65 % respectively. It has been reported in [13] that smoking increases the chances of liver cell cancer. In the previous studies, it was stated that lower risk had been found in former smokers as compared to current smokers, but both groups have a higher risk than those who never smoked [14].

Cirrhosis is a hazardous quotient for the process of hepatic cell carcinoma. The danger is 3–4 times elevated in persons with cirrhosis than in the ones with dreadful hepatitis in a given population. An increase in hepatocellular proliferation may cause enlivening of oncogenes and mutation of tumor suppressor genes. Changes like these may initiate hepatic carcinogenesis [15].

In low-incidence areas, cirrhosis had been reported in more than 90 % of patients with hepatocellular carcinoma. However, in high-incidence areas, the presence of cirrhosis was less (approximately 80 %), in these areas the vertical transmission of hepatitis B virus was common. Whereas, in this study cirrhosis was also the reason for hepatocellular carcinoma.

There are many genetic factors associated with the process as well as the occurrence of tumors which are very complicated and include genomic instability, protooncogene activation, epigenetic alteration, inactivation of the antioncogene, chromosome gain and deletion, and epigenetic alteration [16].

In this study, it was reported that the variant was eliminated with the specific fast reactive enzyme HpyCH4III on the restriction site of PCR products. The same size of heterozygous bands appeared in the affected individuals as in the control group. It was predicted that the GLT-1 allele depicted a greater frequency in HCC persons compared to controls. The small size of the sample made us unable to chalk out a stronger link in the familial HCC group. It was also investigated that the features of other genes, genes and their environmental interactions, epigenetic elements have not been calculated, which may be the staggering variables. Additionally, Matsuura et al. study reported the progression of fibrosis in liver tissue patients that had increased Levels of TLL1 mRNA. The patients had higher rs17047200 AT/TT with gene expression levels of TTL1 short variants, such as isoform 2 [17].

Furthermore, Hong *et al*. (2015) stated that more knowledge could be obtained about the pathogenesis of liver cancer by exploring the susceptibility genes. However, the is not dependent on a single gene. It could be caused due to interactions of mutations in various genes [16].

In this study, we were not able to confirm the significant association between TLL1 genotypes and clinical features of liver cancer patients. Moreover, TLL1 genotypes did not influence HCC features at diagnosis. Several other factors may potentially explain the differences between associations of genotypes in the TLL1 gene with clinical features.

However, it requires further studies that may pay attention to gene-environment interaction as well as gene-gene interactions. So, a better understanding and deepened knowledge of liver cancer may be obtained.

5. CONCLUSION

RFLP on the exon region by using their specific enzymes HpyCH4III showed heterozygous bands in the HCC group that indicated a mutation in the TLL1 gene but the studied genotype was not associated with HCC. So some other factors may potentially affect differences between associations of genotypes in the TLL1 gene with clinical features.

6. CONFLICT OF INTEREST

The authors declared no conflict of interest.

7. ETHICAL APPROVAL

This study was conducted with approval from the Ethics Committee of the Hospitals.

8. PATIENT'S CONSENT

A document of informed consent had signed by all patients.

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Proceedings of the Pakistan Academy of Sciences: Part B
Life and Environmental Sciences 59(2): 71-78 (2022)
Copyright © Pakistan Academy of Sciences
ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(59-2)686

Pakistan Academy of Sciences

Research Article

Antibacterial Potential of *Aloe vera* against *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from Mastitic Milk

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Abstract: The extensive use of antibiotics has developed antibacterial resistance and also may cause toxic effects (hepatotoxicity, nephrotoxicity) on vital organs. To overcome this problem, Scientists gain attention towards medicinal plants. Pure *Aloe vera* (AV) is a common alternative antimicrobial medicine, hence, the current study was conducted to explore its antibacterial potential and compared it with a commonly used antibiotic amoxicillin. During this study, clinically positive mastitis milk samples (n=50) were collected from buffaloes, after microbial culture analysis. Various concentrations (C1=40, C2=20, C3=10, C4=5, C5=2.5, C6=1.25, C7= 0.62, C8=0.31, C9=0.15, C10=0.07 and C11=0.03µl) of pure AV and amoxicillin (µg/µl) were used to evaluate antibacterial activity through minimum inhibitory concentration (MIC) against Gram-positive organisms including *Staphylococcus aureus* and *Streptococcus agalactiae.* The MIC was evaluated based on turbidity and transparency of the medium. Prevalence of *S. aureus* was recorded at 25 (50 %) whereas, 15 (30 %) positive samples for *S. agalactiae* and 10 (20 %) positive samples were found in mixed bacterial colonies from milk samples. The mean values of MIC at 10 µl of pure AV showed 50% sensitivity against *S. aureus* whereas, at 5 µl of pure AV showed 52.5 % sensitivity against S. agalactiae. While amoxicillin inhibited the growth of *S. aureus* and *S. agalactiae* at 2.5 µg/µl and 1.25 µg/µl concentrations showed 52.5 %, and 55 % sensitivity respectively. A significant ($P < 0.05$) difference was noticed between both tested groups. It has been concluded that pure AV possessed antibacterial potential and can be used as a safe and economic alternative against infections caused by *S. aureus* and *S. agalactiae.*

Keywords: *Aloe vera*, Amoxicillin, *Staphylococcus aureus*, *Streptococcus agalactiae*

1. INTRODUCTION

Mastitis is an inflammation of the mammary gland of dairy animals caused by fungi, bacteria and likely viruses [1, 2]. Antimicrobial agents have been used to treat mastitis [3]. Amoxicillin, a broad-spectrum beta-lactam antibiotic commonly administered to treat clinical and subclinical mastitis in cows caused by Enterobacteriaceae, *Escherichia coli, Klebsiella* spp. *Streptococcus agalactiae* and penicillin-sensitive *Staphylococcus aureus* [4, 5]. Since the antibiotic resistance of pathogenic bacteria is increasing rapidly day by day. Scientists get attention towards herbal plants to minimize the antibacterial resistance, among that *Aloe vera* (AV), a medicinal plant that has been used therapeutically for hundreds of years. The center of the AV contains mucilaginous tissue designated as a gel that has been conventionally used for the treatment of a number of ailments such as acne, nourishment for the hairs, gastrointestinal tract disorders, wounds, and sunburn. So far, more than 75 active compounds have been recognized from the AV gel. The gel comprises of 98-99 % water and the remaining 1-2 % containing the active ingredients. It contains a lot of amino acids, antibacterial substances (aloin, polysaccharides, fumaric acid, anthraquinones), vitamins, minerals, enzymes, saponins, sterol, natural sugar, and many other biologically active compounds.

Hence, AV possessed pharmacological

Received: November 2021; Accepted: June 2022
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properties including antimicrobial, anti-inflammatory, antiallergic, antidiabetic, antioxidant, anthelmintic, antifungal, antiseptic, antitumor, protection against radiation, laxative, anti-aging, nephroprotective, and immune stimulation effects [6-8]. The efficacy of crude AV extract has shown a broad range of activity against G+ve and G-ve bacterial organisms. It either kills or inhibits the growth of Streptococcus pyogens, *S. aureus*, *S. agalactiae*, *E. coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Propionibacterium acne*, and *Helicobacter pylori* [9, 10]. AV has been reported to use as a teat-dip or an ointment in lactating cows by intra-mammary administration to treat mastitis or high somatic cell counts [11]. Looking at the scanty information on comparative antibacterial potential, this study was designed to assess the antibacterial activity of AV against *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from mastitis milk of buffaloes and compared it with amoxicillin.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 50 mastitis milk samples of buffaloes were randomly collected (3 & 4 lactating animals) under the aseptic condition in bijous bottles from the buffalo dairy farms in the locality [13].

2.2 Bacteriological Isolation and Identification

The milk samples found positive after the California Mastitis test were examined microbiologically and cultured on Nutrient agar, Blood agar, Edward's agar and MacConkey agar plates. All bacterial growth was identified and recorded after 24 and 48 h of incubation. For the standard, centrifugation, and incubation methods, *S. aureus* were identified by hemolytic pattern, Gram-staining characteristics, positive catalase reaction, positive mannitol agar reaction, coagulase test, Baird-Parker medium culture test, DNase test, Voges-Proskauer test, coagulase test, and annitol fermentation test in positive tube [12]. On the blood agar colony characteristics of *S. agalactiae* were identified morphology appearance of gray to whitish-gray colonies with, beta hemolysis while on Chrome agar it was colony was identified as characteristic light blue color. These findings were confirmed by the growth on the selective media and the positive reaction (Arrow-head formation) to the Christie, Atkins, and Munch-Peterson test.

2.3 Extraction of Gel from *Aloe vera* Leaves

Aloe vera (AV) fresh leaves as shown in figure 1 were collected from a local plant nursery, cleaned with 70 % alcohol, and incised and the gel was separated with the help of a sterile knife. The gel was made homogenous by the process of blending, filtered with muslin cloth and autoclaved at 121 ºC at 15 lb pressure for 15 mins for sterilization. Then, sterilized stock solution (100 % concentration) was used to evaluate antibacterial activity. Sterilized filtrate was diluted as 40, 20, 10, 5, 2.5, 1.25, 0.62, 0.31, 0.15, 0.07 and 0.03 µl [13].

2.4 Preparation of Antibiotic Stock Solution

Amoxicillin stock solution was prepared by adding 15 mg of Amoxicillin powder in 15 ml distilled water then dissolved thoroughly. The solution was sterilized and was kept under refrigeration at 4 ^{0}C until further use. Different concentrations of amoxicillin i.e. 40, 20, 10, 5, 2.5, 1.25, 0.62, 0.31, 0.15, 0.07 and 0.03 µg/µl were used to assess its minimum inhibitory concentration (MIC) [14].

2.5 Determination of Antibacterial Minimum Inhibitory Concentration

For determination of minimum inhibitory concentration (MIC) through microdilution methodology as recommended by Clinical and Laboratory Standard Institute (CLSI) methods with some modifications on Muller-Hinton medium [15]. MIC results were interpreted according to CLSI [16]. 96 well plates containing test microorganisms at the concentration of 2x10^6 CFU/ml [26]. The 96 well plates were incubated at 37 ^{0}C overnight. The break, where the bacterial multiplication was inhibited, was recorded by turbidity/cloudy appearance in cultured wells. These turbidity/ cloudy appearances were recorded as the MIC for pure *Aloe vera* extract and amoxicillin as well.

2.6 Statistical analysis

Data were analyzed by Statistical Package Social Science (SPSS) version 8.1. The experimental results were expressed as mean ± standard error of the mean (SEM). Groups were compared by analysis

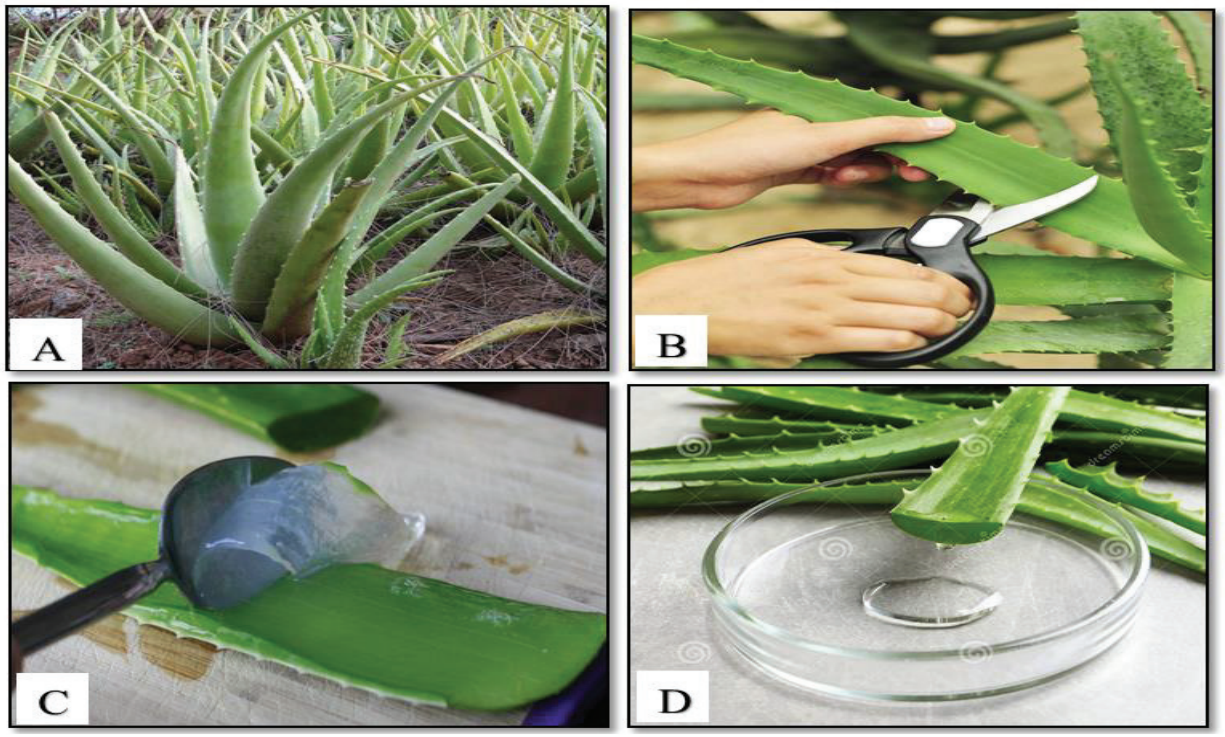

Fig. 1. (A) *Aloe vera* plant, **(B)** Cutting of *Aloe vera* leaves (Source: Google), **(C)** Removal of *Aloe vera* gel from leaves, **(D)** Collection of pure *Aloe vera* gel in petri dishes for antimicrobial activity (Source: Google)

of variance using one-way ANOVA. p-value <0.05 was considered statistically significant.

3. RESULTS

In this study, a total of 50 mastitis milk samples were collected and examined the number and percent prevalence of isolated organisms were recorded. Out of 50 mastitis milk samples, 25 (50 %) were found positive for *Staphylococcus aureus*, 15 (30 %) were positive for *Streptococcus agalactiae* and 10 (20 %) were noticed in mixed colonies.

3.1 Sensitivity of isolated bacteria against pure *Aloe vera* Concentrations

The various concentrations (C1=Control, C2=40, C3=20, C4=10, C5=5, C6=2.5, C7=1.25, C8= 0.62, C9=0.31, C10=0.15, C11=0.07 and C12=0.03 µl) of pure *Aloe vera* (AV) were used for determining the sensitivity of isolated bacterial organisms. It was observed that *Staphylococcus aureus* obtained sensitivity at 40 µl (100 %), 20 µl (87.5 %), and 10 µl (50 %) concentrations, while lower concentrations of pure AV were found resistant against *Staphylococcus aureus*. Whereas Streptococcus agalactiae's growth halted at 40 µl (100 %), 20 µl (100 %), 10 µl (92.5 %) and 5 µl (55 %) concentrations. while lowered concentrations of AV showed resistance against *Streptococcus agalactiae* (Figure 2).

3.2 Sensitivity of Isolated Bacteria against Amoxicillin Concentrations

The various concentrations (C1=Control, C2=40, C3=20, C4=10, C5=5, C6=2.5, C7=1.25, C8=0.62, C9=0.31, C10=0.15, C11=0.07 and C12=0.03 µg/µl) of amoxicillin were used for evaluating its sensitivity against isolated organisms. It was found that *S. aureus* exhibited sensitivity at 40 (100 %), 20 (100 %), 10 (100 %), 5 (80 %) and 2.5 (52.5 %) µg/µl concentrations. Whereas it indicated resistance to amoxicillin below 2.5 µg/µl concentration. While *S. agalactiae* presented sensitivity at 40 (100 %), 20 (100 %), 10 (100 %), 5 (100 %), 2.5 (90 %) and 1.25 (55 %) µl/µg concentrations. But found resistance below 1.25 µg/µl concentrations of amoxicillin (Figure 3).

3.3 Comparative MIC of pure *Aloe vera*, Amoxicillin against Isolated Organisms

The various concentrations (C1=Control, C2=40,

C3=20, C4=10, C5=5, C6=2.5, C7=1.25, C8= 0.62, C9=0.31, C10=0.15, C11=0.07 and C12=0.03µl) of pure AV and amoxicillin were used for halting the growth of isolated organisms. *Staphylococcus aureus* were stop the growth at 10 µl (50 %) concentration of pure AV and 2.5 µl (52.5%) of amoxicillin concentrations. The pure AV stopped the growth of *Streptococcus agalactiae* at 5 µl (55 %) concentrations whereas, amoxicillin exhibited its sensitivity at 1.25 µl (55 %) concentration (Figure 4).

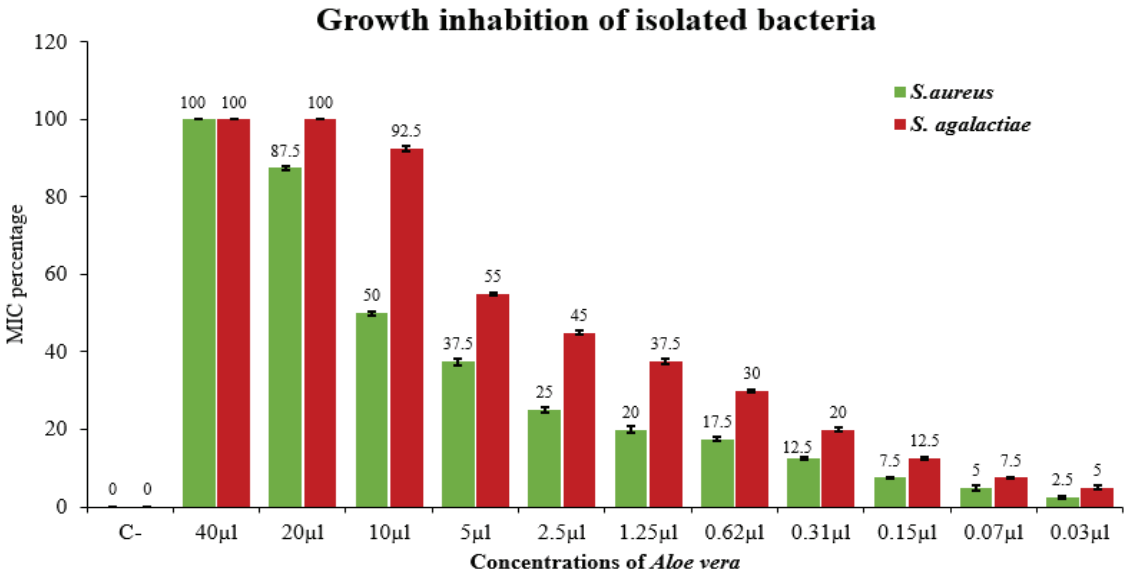

Fig. 2. Minimum inhibitory concentrations (MIC) of antibacterial activity of various concentrations of pure Aloe vera against *S. aureus* and *S. agalactiae*. Significant (P<0.05) difference was determined between both groups.

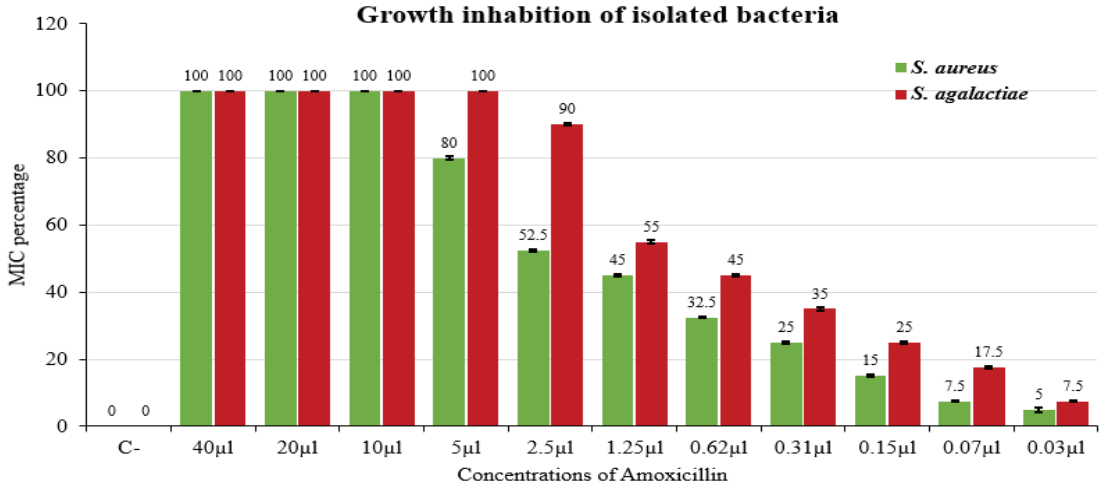

Fig. 3. Minimum inhibitory concentrations (MIC) of antibacterial activity of various concentration of amoxicillin against *S. aureus* and *S. agalactiae*. Significant (P<0.05) difference was determined between both groups

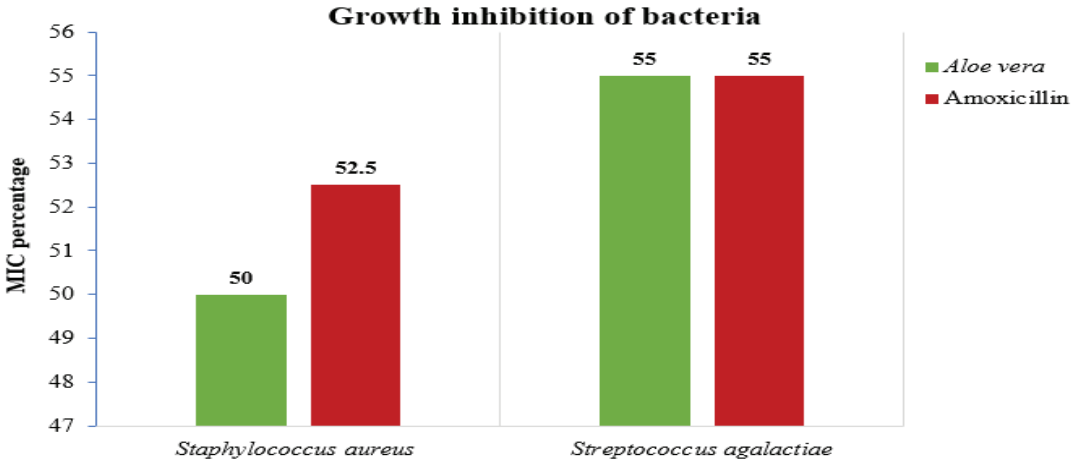

Fig. 4. Comparative minimum inhibitory concentrations (MIC) of isolated organisms against pure *Aloe vera* and amoxicillin. Significant (P<0.05) difference was determined between both groups.

4. DISCUSSION

In the current study, various concentrations of pure *Aloe vera* (AV) and amoxicillin were used to determine their antibacterial activity against *Staphylococcus aureus* and *Streptococcus agalactiae* through the Micro broth dilution method was used to examine minimum inhibitory concentration (MIC) of AV and Amoxicillin against isolated organisms. It was determined that the MIC at which AV inhibited the growth of *S. aureus* and *S. agalactiae* were 20 µl and 5 µl respectively.

The current study showed agreement with previous studies where it was also found that AV extract possessed broad-spectrum antimicrobial activity due to its inhibitory and bactericidal effect against mastitis-causing organisms including *S. aureus*, *S. agalactiae*, *E. coli, P. aeroginosae, P. vulgaris, E. faecalis, S. epidermididis,* and *Bacillus subtilis* [17-19]. The present result is in accordance with previous findings in which crude extract of AV showed antibacterial potential against Gram-positive and Gram-negative bacterial organisms such as *S. aureus*, *S. agalactiae* (Fig. 2) [10, 20]. In these studies, it was observed that the AV gel and leaf possessed antibacterial activity as they inhibited the growth of the above-mentioned bacterial organisms. The AV exhibited antibacterial properties due to the presence of various biologically active ingredients such as carboxy peptidase, emodin, magnesium lactate, salicylate, polysaccharides, anthrone, C-glucosyl chromone, anthraquinones, allantoin dithranol, and chrysarobin [21]. It has also been reported that due to the presence of polysaccharides in the structure of AV, which retained antibacterial activity via the stimulation of phagocyte leucocytes to halt the growth of bacterial organisms [9]. AV also contains the active ingredient anthraquinones which possessed structural similarity with tetracycline antibiotics. Anthraquinone acts like tetracyclines by blocking the 30S ribosomal unit at the accepter side by preventing the transferase enzyme rather than the peptide site. Hence, its mode of action is similar to that of the tetracyclines (where it prevents the access of aminoacyl tRNA to the acceptor site on the mRNA ribosomal complex subsequently, inhibiting protein synthesis of the bacterial organism). It has been reported that the bacterial organisms could not grow in a media containing AV extract [17].

Amoxicillin is a semisynthetic cell wall synthesis inhibitor, it acts by inhibiting the peptidoglycan which is the main polymer of the bacterial cell wall and it is still being administrated continually as a drug of choice within its class [22, 23]. This antibiotic is active against various pathogenic organisms including *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., *Klebsiella* spp., *Shigella* spp., *Trueperella* spp., *Proteus* spp., *Salmonella* spp., *Escherichia* spp. and *Pasteurella* spp. [24]. In the present study, different concentrations of amoxicillin were used to detect the susceptibility of *S. aureus* and *S. agalactiae* isolated from mastitis milk samples of buffaloes (Fig. 3). It was observed that the MIC of amoxicillin for *S. aureus* and *S. agalactiae* was noticed at 10 and

2.5 µg/µl respectively. Furthermore, it was also noticed that *S. aureus* was less susceptible to amoxicillin in comparison to *S. agalactiae* which showed susceptibility even at lowered concentration. This may be owing to selective pressure exhibited in the environment from where the isolates were obtained or it may carry resistant genetic characteristics that showed less susceptibility to amoxicillin concentration.

The present results demonstrated that amoxicillin possessed antibacterial activity against *S. aureus* which is supported by previous findings [4, 25]. However, the present study agreed with the previous study [13] comparison of the various concentration of gentamycin with *Aloe vera* crude extract. The MIC results showed the additive effect with antibiotics, in which growth of E. coli and K. pneumoniae was inhibited at concentrations 1.25 µg/µl and 0.0390625 µg/µl respectively. The present study is also in line with the previously studied comparative study of antibacterial activity of essential oils with antibiotics showed superior effect against the isolated organisms [26]. The present findings are also comparable with previous studies in which antibacterial susceptibility of streptococcus spp. isolated from clinical mastitis samples in dairy cows were reported. It showed that *S. agalactiae*, *S. dysgalactiae, S. uberis* remained susceptible to amoxicillin, while similar findings were noticed by Ikiz *et al*. (2013) and Maia *et al.* (2018) [27, 28]. *S. agalactiae* was found more susceptible as compared to *S. aureus* in the current study, it might be due to less resistance developed in *S. agalactiae* than in *S. aureus* and because of the location of these bacterial organisms in mammary cells. Whereas *S. aureus* inhabits deeper in mammary cells while *S. agalactiae* is localized on the side of the mammary cell. Consequently, during mastitis treatment, antibiotics achieved lowered concentration in deeper mammary cells due to the efflux mechanism that existed in pathogenic organisms. Hence, *S. aureus* received a sub-therapeutic concentration than *S. agalactiae* which would shift the spectrum of activity to an antibiotic to higher concentrations. The bacterial organism may develop resistance through the efflux mechanism, at the target site, which would lower the therapeutic concentration of antibiotics making them less susceptible to the bacterial organisms [29].

5. CONCLUSION

It has been concluded from the current study that the *Staphylococcus aureus* and *Streptococcus agalactiae* are more prevalent in mastitis milk samples in buffaloes. Moreover, both organisms showed susceptibility at slightly higher concentrations against pure AV at different concentrations i.e. 10 and 5 µl but at lowered concentrations then this did not affect the growth of isolated organisms when examined through MIC. On the other hand, amoxicillin retarded the growth of isolated organisms at lowered concentrations than AV. Additionally, amoxicillin also halted the growth of *S. agalactiae* even at the reduced concentration in comparison to *S. aureus* which was inhibited at a higher concentration of the used antibiotic. However, amoxicillin exhibited better inhibition results than pure *Aloe vera* but because of developing antibiotic resistance against commercially available antibiotics and their increased expenses, AV can be used as a safe and economic alternative to amoxicillin against isolated pathogenic organisms.

6. CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest concerning research, authorship, and/or publication of this article.

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Proceedings of the Pakistan Academy of Sciences: Part B
Life and Environmental Sciences 59(2): 79-83 (2022)
Copyright © Pakistan Academy of Sciences
ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(59-2)724

Pakistan Academy of Sciences

Proceedings of the MAAP-PAS-ANSO Hybrid Workshop on "Ecosystem Restoration: One-Health and Pandemics"

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OVERVIEW

Humanity is currently dealing with a number of interlinked existential crises. Ecological degradation, climate change, and biodiversity loss have disastrous consequences for human health and well-being. Furthermore, the emergence and transmission of zoonotic diseases like COVID-19 are linked to ecosystem health. For example, zoonotic infections account for ~75% of new infectious diseases, and they are mainly caused by unsustainable resource usage, animal factory farming, and other large-scale anthropogenic influences. As these pandemics show, environmental destruction can play an important role in a worldwide public-health crisis. It is commonly agreed that COVID-19 will not be the last pandemic. We need holistic approaches like One Health (an area of research that recognizes human, animal, and ecological health as interconnected). One health seeks to increase communication and collaboration between humans, animals, and environmental health professionals to prevent the spread of diseases. To shed light on this important topic, "Ecosystem Restoration: One-Health and Pandemics; hybrid workshop" was organized by the Pakistan Academy of Sciences (PAS) and Monbukagakhusho-MEXT Alumni Association of Pakistan (MAAP); and sponsored by the Pakistan Academy of Sciences (PAS) and Alliance of International Science Organization (ANSO) on June 5, 2022. More than 150 participants attended the hybrid workshop.

1. BRIEF INTRODUCTION

World environment day is celebrated on 5th June every year to bring awareness among the masses about the importance of environmental conservation. This year the slogan for this day was "Only one Earth'' which means that we have only one planet to live on and we need to live sustainably in harmony with nature. To celebrate world environment day, a hybrid workshop on **"Ecosystem Restoration: One-Health and Pandemics"** was organized by the Pakistan Academy of Sciences (PAS) in collaboration with the Alliance of International Science Organizations (ANSO) and MEXT Alumni association of Pakistan (MAAP). The theme of this workshop emphasized on connecting human, animal and environmental health in a balanced way so that none of them is undermined because the human-health is closely connected to the health of animals and our shared environment. The resource persons in the workshop were leading foreign experts from different countries i.e., USA, UK, Japan, China, and Pakistan. They shared their views and their research outputs and discussed development in the area of one health and pandemic. This workshop included two technical sessions.

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2. INAUGURAL SESSION

The Chief Guest of the inaugural session of the hybrid workshop was Prof. Dr. Khalid Mahmood Khan (President, PAS). Moderators of the two technical sessions were Prof. Dr. Muhammad Mukhtar (Vice Chancellor, National Skills University (NSU), Islamabad) and Dr. Shaukat Hameed Khan (Fellow PAS & Ex-Coordinator General COMSTECH).

Prof. Dr. Zabta Khan Shinwari (Chief Organizer, Fellow PAS & President MAAP) was the moderator of the Inaugural Session. Prof. Zabta Khan Shinwari expressed his warm appreciation to the members of the organizing committee, speakers, the Pakistan Academy of Sciences, and especially ANSO for their collaboration to organize this hybrid webinar. Prof. Dr. Tasawar Hayat (Secretary General PAS) welcomed all the guests, speakers, organizers, and workshop participants. He appreciated all the efforts of MAAP, PAS, and ANSO to organize this series of webinars and emphasized on organizing other similar webinars/ events in the future.

In the Inaugural Address, Prof. Dr. Khalid Mahmood Khan (President PAS) shared his views about the importance of a healthy ecosystem and that one health has changed the conventional approach that only human health is of prime importance. He supported this idea by sharing the fact that a major portion of human infectious diseases are of zoonotic origin. Environmental changes and anthropogenic factors are exacerbating the problem of ecological imbalance. He highlighted the challenges and loopholes in managing the current pandemic (Covid-19) and advocated the need for strong collaboration among the scientific community and state institutions.

3. TECHNICAL SESSION I

Technical Session I was chaired by **Prof. Dr. Muhammad Mukhtar (Vice Chancellor, National Skills University, Islamabad).** The first speaker, **Prof. Dr. Zabta Khan Shinwari** briefly explained "Biodiversity loss: One health and pandemic." He elaborated the grave concern of biodiversity loss and stressed on its conservation by quoting "No one is safe until everybody is safe"; which highlights the importance of timely holistic actions to regulate animal trade and conserve wildlife biodiversity. He also considered Covid-19 a blessing in disguise as it has given us a wake-up call to take measures for environmental protection so that we could avoid such lethal pandemic(s)

Fig. 1. Participants of Inaugural Session of MAAP-PAS-ANSO Hybrid Workshop on Ecosystem Restoration, One-Health and Pandemic with the Prof. Dr. Tasawar Hayat (Secretary General PAS), Prof. Dr. Muhammad Mukhtar (Vice Chancellor, National Skills University (NSU), Islamabad), Dr. Shaukat Hameed Khan (Fellow PAS & Ex-Coordinator General COMSTECH), and Prof. Dr. Zabta Khan Shinwari (Chief Organizer, Fellow PAS & President MAAP) organized by the Pakistan Academy of Sciences (PAS) and Monbukagakhusho-MEXT Alumni Association of Pakistan (MAAP); and sponsored by the Pakistan Academy of Sciences (PAS) and Alliance of International Science Organization (ANSO)

again, or at least could prepare ourselves for any environmental degradation of a lesser degree.

Prof. Nancy Connell (US-NAS) (The National Academy of Sciences, USA) introduced a very emerging domain of technology Artificial intelligence (AI) that can be incorporated into the field of biological conservation. The topic of the talk was "AI and biodiversity". As she started with a very powerful quotation by E O. Wilson "If there is danger in human trajectory, it is not so much in the survival of our species as in the fulfillment of the ultimate irony of organic evolution: that in the instant of achieving self-understanding through the mind of man, life has doomed its most beautiful creations." She presented the highlights of her research project that how they have used artificial intelligence to monitor and regulate biodiversity, and also in the improvement of conservation and sustainable use of biological and ecosystem values in a rapidly changing and resource-limited world.

Dr. David R. Franz (US-NAS) elucidated the importance of insurance policy for the future in a rapidly changing and complex world to alleviate the effects of future pandemics. He stressed the idea by saying that "the planning is more important than the plan". The topic of his presentation was "Insurance policy for the future" he mainly elaborated the drawback of human behavior that forgets the harsh past without doing anything to save the future generation. His presentation mainly consists of how we can focus on the one health concept that can improve the living conditions of both humans and animals with aid of science and technology. Lastly, he emphasized imposing a national strategy to countermeasure any future pandemic.

Dr. Tim Trevan (Co-founder, Chrome Biorisk management consulting) talked about "Zoonosis: Can we mitigate risks species jumps". He stated that zoonosis is the evolution in action, and evolution requires replication that is further enhanced by selective pressure. In his lecture, he explained the stages of viral replication such as attachment, penetration, synthesis-coating, release, genomic assemblage, and finally viral protein synthesis. Further, he stated that there is no evolution possible without selective pressure, and no selective pressure is possible without replication. Such evolution and selective pressure can lead to pathogen spillover and viral propagation.

Dr. Niryoshi Shinomiya (President, National Defense Medical College, Japan) talked about the "Advances in the life sciences and the risk of pandemics". He also put forward the highlights of research projects on the possibility of artificial synthesis of various viruses and expressed concern that the eradicated viruses such as the smallpox virus can reemerge due to its chemical synthesis. Prof. Niryoshi Shinomiya also highlighted the need for and importance of biosafety and biosecurity with the installation of biological safety levels in labs worldwide. His presentation further contains a timeline of the human warfare threats that shows traditional agents during the 1970s, genetically modified traditional agents during the 1990s, and synthetic biology recombinant genetics and genome editing after the 2000s. Additionally, he also illustrated trends in life science technologies from the past twenty years and emphasized on regulating technologies like genome editing, etc.

4. TECHNICAL SESSION II

Technical Session II was chaired by **Dr. Shaukat Hameed Khan (Fellow PAS & Ex-Coordinator General COMSTECH).** The first speaker of the second session was **Dr. Muhammad Ali (Principal investigator, ANSO Project & Assistant Professor, Quaid-i-Azam University Islamabad)** who gave a brief overview of "Bats viruses and Pandemics". He discussed that bats are one of the largest reservoirs of mammalian viruses due to their migratory behavior, seasonal hibernation & roosting, altered antiviral immunity, and unique anti-inflammatory and proinflammatory responses. Some of the bat-borne viruses have spread to the human population by crossing specie-barriers. He gave an overview of global ongoing research on bat viromes and discussed current challenges. In concluding remarks, he emphasized the need for close and apolitical collaborations among scientists in combating pandemics.

Dr. Qadeer Ahsan (Fleming fund) discussed the pandemics with respect to Pakistan. He emphasized on that antimicrobial resistance aggravated the covid-19 pandemic situation. He discussed the role of the Fleming Fund to improve the AMR surveillance and support a national

action plan to transform policy and delivery of healthcare in Pakistan. He also discussed the clinical stewardship program initiated by the fleming fund and highlighted its main objectives. He also emphasized on laboratory infrastructure enhancement, federal and provincial interventions, system development, and disease surveillance. He also mentioned the results of the knowledge attitude and practice (KAP) survey.

Dr. Quaid Saeed (CEO, Islamabad Healthcare Regulatory Authority; IHRA) shed light on the role of IHRA in the management of the Covid-19 pandemic. In his message, he discussed different strategies that were adopted by them to cope with emergencies. He also discussed that health regulatory authorities like IHRA are under pressure during the pandemics for taking urgent and important decisions regarding controlling the pathogen, vaccine approvals/strategies, and regulating healthcare-related issues. IHRA managed the complaints from the masses regarding malpractices by the hospitals and diagnostics facilities.

Prof. Dr. Li Cui (Institute of Urban Environment, CAS) briefly explained her research projects on antimicrobial resistance and one health. She focused on the environmental dimension of antibiotic resistance and reservoirs of AMR that are neglected somehow. Initially, she explained the general concept of antibiotic resistance, followed by the severity of global antimicrobial resistance and how it leads to a silent pandemic by slowly ending millions of lives worldwide. she elaborated that one health is essential not only to avoid any future pandemic but also to the major threat of antibiotic resistance. She stated that increased contact of humans with wild animals can enhance antibiotic resistance and transfer these into the food chain which can affect the primary and secondary consumers in the food chain.

Following **Dr. Li Cui, Dr. Shahbaz Khan (UNESCO, Beijing)** presented the concept of open science for all-leaving no one behind. The implementation of this idea needs a transparent, inclusive, democratic, and sustainable approach. Research findings should be more accessible, and for that, we need open communication, open knowledge, shared research, infrastructure, open labs, crowdsourcing, crowdfunding, etc.

Lastly **Prof. Lijun Shang and Prof. Malcolm Dando** (London Metropolitan University, UK) talked about the topic "Is there sufficient educational resources for the implementation of Tianjin Biosecurity guidelines for codes of conduct for life scientist result and survey" in their presentation of some of the backgrounds that leads to the development of the Tianjin biosecurity guidelines. That includes education and training and how it is promoted by Pakistan and China relationship.

5. CONCLUDING SESSION

The Chief Guest of the concluding session was **H.E Ryuji IWASAKI,** Counsellor, **Embassy of Japan** in Pakistan with a take-home message on the importance of environmental preservation and how it is essential for us, and how by adopting Ecosystem restoration: One health and pandemic approach to not only prevent outbreaks in zoonotic diseases, but also the other environmental issues including food safety and antimicrobial resistance. Furthermore, it's a collective responsibility of all different government and non-government organizations to address the challenges through the engagement of society and the research community along with the introduction of new policies to mitigate these threats for the future generation.

In total, 11 lectures were presented by eminent local and foreign speakers in two technical sessions of the hybrid workshop while a concluding message was presented on behalf of Dr. Fazal Hadi, Chair Board, Islamabad Healthcare Regulatory Authority by Dr. Shaukat Hameed Khan (Fellow PAS & Ex-Coordinator General COMSTECH).

"The health of the planet impinges on all the living creatures, flora, and fauna. Recent events have proved that we are knowingly and unknowingly damaging the environment of our planet at a fast pace. We are nearly on the brink of no return. There is hardly any time left. Very urgent steps are needed on a war footing. The industrialized world is largely responsible. It has a voracious appetite for the resources of the world, which are finite. They know this but continue their quest for fast profits. They think they will somehow escape. They will not be able to! We the present generation on this planet are

Fig. 2. Participants of Concluding Session of MAAP-PAS-ANSO Hybrid Workshop on Ecosystem Restoration, One-Health and Pandemic with the Chief Guest H.E Ryuji IWASAKI, Counsellor, Embassy of Japan in Pakistan, Prof. Dr. Tasawar Hayat (Secretary General PAS), Dr. Shaukat Hameed Khan (Fellow PAS & Ex-Coordinator General COMSTECH), and Prof. Dr. Zabta Khan Shinwari (Chief Organizer) organized by the Pakistan Academy of Sciences (PAS) and Monbukagakhusho-MEXT Alumni Association of Pakistan (MAAP); and sponsored by the Pakistan Academy of Sciences (PAS) and Alliance of International Science Organization (ANSO)

leaving a terrible legacy for our future generations, whose fate is in serious jeopardy anyway. Outbreaks of new diseases, never seen before, are on the increase. Pandemics are becoming common. The human and financial costs of these cannot be estimated in real terms. Human greed bent upon the destruction of a hitherto untouched and pristine environment is playing havoc with the lives of all living beings. The only way to stop all this is to get together again and again to devise ways and means and to monitor them, to put a stop to this madness of wanton destruction of our planet, the only one we have."

The session was concluded by Prof. Dr. Tasawar Hayat (Sec. Gen. PAS). He expressed his gratitude to worthy Chief Guest H.E Ryuji Iwasaki for sparing his precious time to grace this Workshop. In addition, a few posters were also presented by participants of the workshop. "For this, I am indebted to all the speakers of the workshop for their thought-provoking lectures on a broad range of topics in line with the themes of the workshop and for fruitfully sharing their knowledge and expertise for the benefit of the participants of the workshop. We must, therefore, give a big round of applause to all the local and foreign faculty members and poster presenters" he added. Secretary General also appreciated and acknowledged the speakers, moderators, organizers, and participants of the workshop and especially thanked ANSO for its financial support. To acknowledge the contributions of the speakers, the PAS presented Souvenirs to those speakers and moderators who were physically present at the venue.

6. CONCLUSION

This workshop provided a platform for a wholesome discussion on ecological restoration and presented some robust solutions and conceptual frameworks that could be very helpful to address the existing ecological crises. One health approach can be a very effective strategy to cope with the disastrous effects of an imbalance between humans and the environment and deadly pandemics that sprout from the critical nexus of human beings and the ecosystem.

7. ACKNOWLEDGEMENT

Authors are thankful to Alliance of International Science Organizations (ANSO) for funding under the ANSO collaborative research grants (ANSO-CR-PP-2021-05).

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3. R.K. Robert, and C.R.L.Thompson. Forming patterns in development without morphogen gradients: differentiation and sorting. *Cold Spring Harbor Perspectives in Biology* 1(6) (2009).
4. D. Fravel. Commercialization and implementation of biocontrol. *Annual Reviews of Phytopathology* 43: 337359 (2005).

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5. W.R. Luellen. Fine-Tuning Your Writing. *Wise Owl Publishing Company, Madison, WI, USA* (2001).
6. U. Alon, and D.N. Wegner (Ed.). An Introduction to Systems Biology: Design Principles of Biological Circuits. *Chapman & Hall/CRC, Boca Raton, FL, USA* (2006).

c. Book Chapters

7. M.S. Sarnthein, and J.D. Stanford. Basal sauropodomorpha: historical and recent phylogenetic developments. In: The Northern North Atlantic: A Changing Environment. P.R. Schafer, & W. Schluter (Ed.), *Springer, Berlin, Germany*, pp. 365–410 (2000).
8. J.E. Smolen, and L.A. Boxer. Functions of Europhiles. In: Hematology, 4th ed. W.J. Williams., E. Butler and M.A. Litchman (Ed.), *McGraw Hill, New York, USA*, pp. 103–101 (1991).

d. Reports

9. M.D. Sobsey, and F.K. Pfaender. Evaluation of the H2S method for Detection of Fecal Contamination of Drinking Water, Report WHO/SDE/WSH/02.08, *Water Sanitation and Health Programme, WHO, Geneva, Switzerland* (2002).

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