NEPAL JOURNAL OF SCIENCE AND TECHNOLOGY

Vol.22(1)
NEPAL ACADEMY OF SCIENCE AND TECHNOLOGY
KATHMANDU, NEPAL

Nepal Journal of Science and Technology (NJST) Board Members

NEPAL ACADEMY OF SCIENCE & TECHNOLOGY
CENTRAL OFFICE
NAST

Message from the Editor-in-Chief

Warm greetings from the Editor-in-Chief

I take pleasure in welcoming you all to the volume 22(1) of Nepal Journal of Science and Technology (NJST), published by Nepal Academy of Science and Technology (NAST). NJST is a peer-reviewed journal that publishes twice a year, incorporating research, review, perspective and communication manuscripts on applied and natural sciences.

NJST has been able to mark its credibility but the improvements are far from satisfactory. We are determined to make it one of the leading journals in future. NJST embraces broader spectrum of S & T and hence, welcomes multidisciplinary manuscripts, which undergo thorough review. In this issue we proudly present 16 articles from the international and national contributors who have shared their research findings on different arena of science and technology. I take this opportunity to thank the authors for choosing NJST to publish their findings and making the journal prestigious.

I extend my sincere gratitude to the NJST advisory board, editorial board, managing editor, associate managing editor, and the whole team of publication for their unlimited support, and for guiding the journal to greater heights. Special appreciation goes to the reviewers for their meticulous contribution towards reviewing the manuscripts.

Prof. Dr. Dilip Subba
Editor-in-Chief
Nepal Journal of Science and Technology (NJST)
Nepal Academy of Science and Technology (NAST)

Table of Content

Content *Pages*

Immature Production of Dengue Virus Vectors in Residential and Non-residential Areas of Lalitpur Municipality, Nepal

Aradhana KC[1], Manchita Kumari Aryal[2], Ishan Gautam[3*] and Hans Jorgen Overgaard[4]

[1]Save the Children International, Kathmandu, Nepal
[2]Nepal Academy of Science and Technology, Lalitpur, Nepal
[3]Natural History Museum, Tribhuvan University, Kathmandu, Nepal
[4]Norwegian University of Life Sciences, Ås, Norway

***CORRESPONDING AUTHOR:**

Ishan Gautam
Email:ishan.gautam@nhm.tu.edu.np

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI : https://doi.org/10.3126/njst.v21i2.67154

Date of Submission: February 22, 2023

Date of Acceptance: July 22, 2023

ABSTRACT

Global incidence of dengue has grown over recent decades, with half of the population now at risk. Vector control is the main way to control dengue disease, as many dengue vaccines are still under research. *Aedes aegypti* and *Aedes albopictus* are the vector species, responsible for dengue transmission in the world.

The repeated larvae and pupae sampling of eight times in dry and wet seasons (May to September) was conducted within 100 houses including residential and non-residential. Dipping method using standard dippers were used for immature mosquito collection.

This study found that non- residential areas are preferred breeding sites for dengue mosquitoes compared to residential premises. The *Stegomyia* indices, House Index (HI), Container Index (CI) and Breteau Index (BI) were found higher in non-residential houses than that of residential houses.The statistical analysis shows strong significant differences, $p<0.05$ when compared between two seasons (dry and wet). Seven different types of containers classified by shape, use and materials contribute 72-74% of immature dengue mosquitoes. This study concludes that for dengue mosquitoes' production, dark coloured containers found in both residential and non-residential sites are highly productive. Thus, further studies covering all seasons and households are highly recommended in the study sites leading to effective vector control actions targeting all types of productive wet containers available in the study area and elsewhere.

Keywords: Dengue, Immature, Mosquitoes, Non-residential, Residential

1. Introduction

Aedes-borne diseases including dengue, chikungunya and zika are a growing problem worldwide. Dengue fever, in particular, has increased 30-fold, extending its range in new countries, from urban to the rural areas, in the past 50 years (Gubler 1998; WHO 2008; WHO 2009). It is one of the fastest-growing global infectious diseases, with 100–400 million new infections each year (Brady & Hay 2020) and an estimated 3.83 billion people living in areas suitable for dengue transmission (Messina *et al*. 2019). Additionally, 96 million people with dengue infections were recorded globally in 2010, of which 70% were from Asia. Among this 34% were recorded from India alone (Bhatt *et al*. 2013).The disease is further classified into three types, classical dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Hadinegoro 2012). A small single stranded RNA virus of genus Flavivirus and family Flaviviridae causes dengue fever, which consist of four serotypes, DENV 1, DENV 2, DENV 3, and DENV 4 (WHO 2009). Dengue fever was first recorded in Nepal in 2004 from Chitwan district (Pandey *et al*. 2004). The outbreak of dengue fever started in Nepal from 2006, which was recorded from nine districts of low land region of Nepal (Pandey *et al*. 2008; Malla *et al*. 2008). More cases of dengue from Kathmandu valley were recorded from dengue outbreak in 2010 (Pandey *et al*. 2013) and all four dengue virus serotypes are expanding their range in new geographical areas of the country, Nepal, which will further increase the risk of dengue outbreaks in new areas (Pun 2011).

The virus is transmitted through the bite of *Ae. aegypti* and *Ae. albopictus* (Gubler 2002; Gratz 2004; Ponlawat & Harrington 2005; Bonizzoni *et al*. 2013). Both species coexist in Nepal. *Ae. aegypti* is considered the principal vector of dengue. On the other hand, *Ae. albopictus* alone has been confirmed as the vector in some dengue outbreak areas (Paupy *et al*. 2009). It has also driven the global emergence of chikungunya virus in as well (Weaver & Forrester 2015). Anthropogenic changes such as urbanization, alterations in land use, increased cross country trade, travel networks and vehicular movement, climate change etc. have impacted their distribution and geographical expansion (Kolimenakis *et al*. 2021).

Ae. albopictus originated in the forests of Southeast Asia (Paupy *et al*. 2009) and first documented in 1956 in Nepal including Halchowk, Kathmandu (Peters & Dewar 1956). Though, no scientific publication came through regarding the presence of *Ae. aegypti* in Nepal until 2006 when this species was recorded in the selected urban settings of different districts of Terai region near Indian border namely Morang (Biratnagar), Parsa (Birgunj), Chitwan (Bharatpur), Dang (Tulsipur) and Banke (Nepalgunj) (WHO 2006; Malla *et al*. 2008). In Kathmandu valley, *Ae. aegypti* was reported in the year 2009 for the first time (Gautam *et al*. 2009). Both species are expanding its geographical range up to an altitude of 1,350 m and sparsely in 1,700 to 2,100 m in Nepal and distributed in sub- tropical regions (Dhimal *et al*. 2015) including Lalitpur district of Nepal (Gautam *et al*. 2009).

Ae. aegypti have become widely distributed in tropical regions of the Asian, South American, and African continents and *Ae. albopictus* is commonly found in most of the countries of Asia, Africa, America, and Europe (WHO 2009; Braks *et al*. 2003). Common breeding habitat for *Aedes aegypti*is in artificial containers with clear water, where as *Aedes albopictus* prefer to breed in natural water holding containers (Christophers 1960; Bonizzoni *et al*. 2013). The eggs of these species can survive in adverse climatic conditions like long winter and droughts (Sota & Mogi 1992). The larvae of both species feed on microorganisms, organic detritus and other food particles found in the water holding containers (Braks *et al*. 2004). Adult stages of these species are aggressive day biting mosquitoes with bimodal biting behaviour. *Ae. aegypti* has peak biting period at dawn and dusk and *Ae. albopictus* biting peaks from 06:00-09:00 to 15:00-20:00hr GMT (Ho *et al*. 1973; Chen *et al*. 2014). The adult female feed on human blood and disperse for food, oviposition and searching for mate. Dispersal for oviposition of this mosquito is pertinent for the disease propagation (Lambrechts *et al*. 2010; Muir & Kay 1998; Honorio *et al*. 2009). Female *Ae. aegypti* is highly anthropophilic in nature and well adapted in urban areas (Ponlawat & Harrington 2005). While *Ae. albopictus* has adapted to anthropogenic changes in the environment, feeding more frequently on humans and domestic animals, although it remains more abundant in vegetated rural and suburban areas (Hawley 1988). Density is high when there is greater population of human settlements with low socioeconomic status (Tauil 2001). The size and the biological status are the determinant to transmission dynamics of the disease. Rainfall, high temperature, high humidity, and moisture are the important drivers of vector reproduction and also help to enhance the vectorial capacity. Additionally, temperature also affects the gonotrophic cycle and survival of the primary vector of dengue (Yang *et al*. 2009).

The classical *Stegomyia* indices show the absence or presence of the vector. Pupal productivity surveys are a much better representative indicator for adult vector abundance in dry and/or inwet season because the total number of *Aedes* pupae is used as a proxy indicator for adult dengue vector density, as roughly 80% of pupae develop to adult mosquitoes (Focks & Alexander 2006). Additionally, it explicitly depicts the most productive *Aedes* water container types in the dry and the wet seasons coupled with variation of the pattern among different residential or non-residential settings leading to targeted management of the most mosquito-productive containers for eliminating all potential breeding habitats in various socio-ecological settings. Abundance of immature dengue mosquitoes were found higher in non-residential areas compared to residential areas (Baak-Baak *et al*. 2014). Dos *et al*. (2010) also argued that the study on dengue vectors in Brazil shows that non-residential sites were key sites for vector surveillance than that of residential areas. The pupal demographic survey of *Ae. aegypti* in non-residential areas of Peruvian city of Iquitos shows that such areas are highly productive compared to residential areas (Morrison *et al*. 2006).

Vector control is the main way to control dengue, as many dengue vaccines are still under research (Jacobs 2000; Koenraadt *et al*. 2007; Deng *et al*. 2020). Some other methods are spraying larvicides, introducing predatory fish in water holding containers etc. (Baak-Baak *et al*. 2014; Dos *et al*. 2010; Kroeger *et al*. 2006). Although, it is necessary, at this juncture, to conduct larval and pupal-demographic surveys which pave a path toward effective methods for vector control (Ponlawat *et al*. 2005; Nathan *et al*. 2006) through eliminating mosquitoes breeding containers from residential and non-residential areas.

Different studies on dengue virus and vector surveillance have been conducted previously from lower tropical and sub-tropical regions including container preference of *Ae. albopitus* in Kathmandu and Lalitpur district (Gautam *et al*. 2012). However, most of the dengue vector surveillance was only focused on residential sites often neglecting non-residential sites, which might be potential breeding sites in large volume for dengue mosquitoes. Furthermore, there is a lack of studies on breeding site characteristics and immature dengue mosquitoes' production in residential and non-residential areas.Keeping this in mind, this study aims to compare immature production of *Ae. aegypti* and *Ae. Albopictus* as well as to find out the most productive containers in residential and non-residential areas of Lalitpur district of Nepal.

2. Methods:

2.1 Study area:

Two wards of Patan city of Lalitpur district, Nepal were chosen for the study. Lalitpur sub-metropolitan city is located between N 27 °39'' and E 27° 41'', with the elevation of 457 m to 2831m above sea level. The city lies near to capital city of Nepal (Kathmandu). It is one of the oldest cities of Nepal which consist of old houses and historical places (Fig. 1).

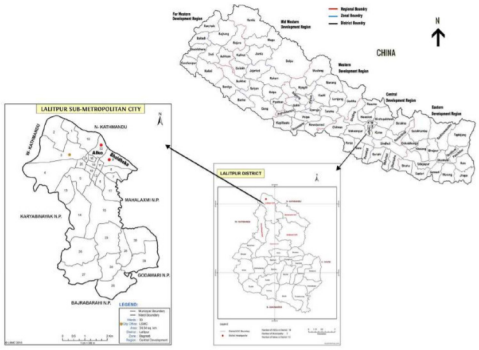


Fig. 1: Map of Study Area

2.2 Study design and sampling:

A series of cross-sectional entomological survey with repeated sampling in May-June (dry season) and August-September (wet season), 8 times for 4 months period, and 2 surveys in each month was performed in residential and non-residential areas of the city in altitudes ranged from 1200 to 1300m. Hundred houses were randomly selected covering both areas from two wards (Aliko and Bholdhoka) of the city. A team of 5 persons were employed to conduct larval and pupae surveillance from 7 to 11 am. The non-residential areas include cement block factories, mud statue factories, metal workshops, tire repair shops, temple, furniture factories, government and private offices, rice mills, electronic shops, restaurants, garbage recycling centres, kindergarten and schools and grocery shops.

Oral informed consent was taken from the head of the each household before starting the collection of larval mosquitoes. In case where the household head

disagreed, the house was dropped from the collection plan and the immediate next one was selected for survey.

2.3 Entomological Survey:

All the water holding containers from the residential and non-residential areas were screened for the presence and absence of immature *Aedes* mosquitoes and were collected by using standard larvae collection procedure (Su *et al*. 2016). The containers were inspected using flashlight. Immature mosquitoes were collected using dippers of different size and pipettes (Vikram *et al*. 2016). All larvae and pupae were transferred to plastic bags and labelled with house code, container code, date and locality. According to Koenraadt *et al*. (2007), all the wet containers were recorded based on the shape, use and materials (SUM) method. Other associated variables include, presence of cover (yes or no), location (indoor or outdoor), size (length, height and opening), water depth, shade (yes, no or partially), under roof (yes, no or partially), water source (rain fed or manually), insecticide used (yes or no) and container washed (yes or no). There was no active vector control method applied in the area during surveillance. Weekly or monthly temperature was not included in the analysis.

2.4 Laboratory work:

All the collected larvae and pupae were brought to the laboratory at the Natural History Museum, Kathmandu, Nepal for rearing and identification and transferred to plastic cup and covered with thin muslin cloth and rubber bands. Plastic cups were kept in the laboratory under normal temperature conditions for rearing. The labelled plastic cups were checked once a day for adult emergence. Adult were then transferred to test tubes by using aspirator and killed with chloroform. Larval mosquitoes that did not emerge to adult were preserved in 70% alcohol in vials and prepared slide. Adult mosquitoes were identified to species level by using taxonomic keys, dissecting microscope, hand lenses (10X triplet hand lens) and pointed forceps. The slides containing larvae were observed under compound microscope and identified using the standard keys (Darsie & Pradhan 1990; Rueda 2004; Fenemore 2006).

2.5 Data analysis:

Traditional *Stegomyia* indices were used to evaluate the population densities of the dengue mosquitoes in residential and non-residential areas, such as house index (HI), container index (CI) and Breteau index (BI). These techniques were commonly used as standard parameters in most of the developing countries (Petric *et al*. 2014).

Findings of the survey were analysed using Microsoft Excel 2013 spread sheet and SPSS version 21. Descriptive analysis was conducted to carry out the container characteristics, immature mosquitoes'infestation, and percentages in residential and non-residential areas. Container productivity of *Aedes aegypti* and *Aedes albopictus* were classified by shape, use and material and ranked from highest to lowest. Negative binomial regression model to test the significance difference between two areas at 95% confidence level for both species was carried out using SPSS.

3. Results

3.1 Container characteristics:

Of the 1779 wet containers, 1259 from residential areas and 520 from non-residential areas were screened covering 694 outdoor and 565 indoor locations and 332 outdoor and 188 indoor locations of residential and non-residential areas respectively (Table 1). The size of the container and water depth in both areas are shown in Table 2.

Table 1. Container characteristics in residential and non-residential areas of Lalitpur district, Nepal.

	Residential areas (n - 68)	%	Non-residential areas (n - 32)	%	Total (n)	Total (%)
Number of wet containers	1259	71	520	29	1779	100
Location						
Outdoor	694	68	332	32	1026	58
Indoor	565	75	188	25	753	42
Cover lid						
Yes	615	76	190	24	805	45
No	644	66	330	34	974	55

Filling method						
Rain	439	65	232	35	671	38
Manual	820	74	288	26	1108	62
In shade						
Yes	385	73	142	27	527	29
Partially	97	63	57	37	154	9
No	777	71	321	29	1098	62
Under roof						
Yes	735	74	260	26	995	56
Partially	22	56	17	44	39	2
No	502	67	243	33	745	42
Wash before refill						
Yes	715	76	229	24	944	53
No	544	65	291	35	835	47

Table 2. Size of the container and water depth.

	Residential	Non-residential
Maximum		
Length (cm)	200	200
Width (cm)	100	100
Height (cm)	250	250
Opening (cm)	200	200
Minimum		
Length (cm)	5	5
Width (cm)	2.5	2.5
Height (cm)	5	8
Opening (cm)	4	5
Average (cm)		
Length (cm)	41.9	46
Width (cm)	20.5	22.37
Height (cm)	56.9	57.3
Opening (cm)	40	44.7
Water depth (cm)		
Maximum	197	206
Minimum	1	1
Average	38.3	37.7

3.2 Shape, use and material:

The main container types were drums (793), buckets (504), pots (270), tanks (94), gallons (55), tires (42) and jars (21). Among these, 72% of the drums were in residential houses and 28% in non-residential houses. Of the buckets, 76% were in residential houses and 24% in non-residential houses. The corresponding residential and non-residential Fig.s for pots were 68% and 32%, for tanks 56% and 44%, for gallons 76% and 24%, and for jars 67% and 33% respectively. Pots were made up of either metal and plastic or clay, and drums were either plastic or metal. Most of the plastic drums were black, yellow and blue in colour, whereas metal drums were blue or brown. Buckets were made from plastic or metal, tires from rubber, and tanks from cement. Most of the plastic pots were used for washing such as hand and face washing, brushing, and cleaning. No use of metal pots in non-residential areas was observed, whereas metal pots were used for irrigation in gardens and drinking water for pets in residential areas. Clay pots in residential areas were used for ornamental flowers. Drums, buckets and cement tanks were found to be used for daily washing propose (dishwashing, bathing, cooking and clothes washing). Large cement tanks were used for all types of washing and drinking and cement tanks in non-residential areas were used for making statue, cement blocks and rings in non-residential houses. Jars and gallons were used to store drinking water. Discarded tires were found lying outdoor near non-residential houses such as workshops, repairing shop, recycling centre etc.

3.3 Mosquito immature infestation:

A total of 136 containers (136/1779 = 7.6%) were infested with *Ae. aegypti* larvae and pupae. These were

pots (n = 58), drums (n = 42), buckets (n = 24), tires (n = 10), and tanks (n = 2). For *Ae. albopictus* all together 152 containers (152/1779=8.5%) were found positive for larvae and pupae. These were pots (n = 62), drums (n = 43), buckets (n = 37), tires (n = 9) and a tank (n = 1). A total of 122 containers (6.9%) were infested with *Culex* spp; these were drums (n = 44), pots (n = 32), buckets (n = 23), tanks (n = 18), and tires (n = 5). Thirty-four containers (2%) were positive for other *Aedes* mosquito pots (n = 14), drums (n = 10), buckets (n = 7), tires (n = 2) and tank (n = 1).

3.4 Mosquitoes in residential and non-residential areas:

All together 2107 larvae and pupae were recorded from the whole survey, of which 484 were *Ae. aegypti*, 304 from the residential land 180 from the non- residential sites.Whereas776 *Ae. albopictus*, 479 from residential and 297 from non-residential (Table 3). Most abundant species was *Ae. albopictus* (n = 776), and then *Culex* spp. (n = 713), followed by *Ae. aegypti* (n = 484), other *Aedes* species (n = 96), *Anopheles* species (n = 24) and other unidentified mosquitoes were 14.

Table 3: Number and proportion of immature mosquitoes collected in residential and non-residential areas in May, June, August, and September 2016.

Species	Residential		Non-residential		Total	
	Number	%	Number	%	Number	%
Aedes aegypti	304	63	180	37	484	100
Larvae	209	63	121	37	330	100
Pupae	95	62	59	38	154	100
Aedes albopictus	479	62	297	38	776	100
Larvae	264	61	169	39	433	100
Pupae	215	63	128	37	343	100
Anopheles spp.	12	50	12	50	24	100
Culex spp.	448	63	265	37	713	100
Aedes spp.	64	67	32	33	96	100
Unidentified	6	43	8	57	14	100
Total	1313		794		2107	

3.5 The *Stegomyia* indices by areas:

The House Index, Container Index and Breteau Index for immature dengue mosquitoes was higher in non-residential houses than in residential houses (Table 4).

Table 4. The *Stegomyia* indices of *Aedes aegypti* and *Aedes albopictus* in residential and non-residential areas.

	Area		
	Residential	Non-residential	Total
Total no. of wet containers encountered	1259	520	1779
Average no. of wet containers per house	2.3	2.1	2.2
Number of positive houses	102	63	165
Number of positive containers	110	65	175
Container Index (CI)	8.7	12.5	9.8
House Index (HI)	18.8	24.6	20.6
Breteau Index (BI)	20.2	25.4	22
Number of pupae positive containers	71	39	110
Total number of pupae	310	187	497
Pupae per house index (PHI)	57	73	62

CI = Percentage of water holding containers infested with immature dengue mosquitoes.

HI = Percentage of houses infested with immature dengue mosquitoes.

BI = Number of dengue mosquito positive containers per 100 houses.

PHI = Number of pupae per house.

3.6 Seasonal distribution of immature mosquitoes:

In the dry season the highest number of mosquitoes recorded was of *Culex* spp., the second highest was *Ae. albopictus*, followed by *Aedes aegypti* and then other *Aedes* spp. (Fig. 2). For the post-monsoon season the most abundant mosquitoes was *Ae. albopictus*, and *Ae. aegypti* followed by *Culex* spp. (Fig. 2).

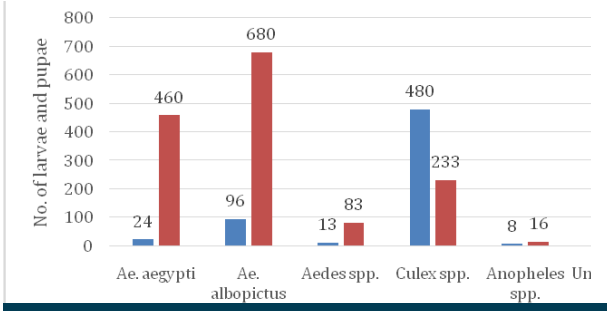


Fig. 2: Total larvae and pupae collected in dry (May-June) and wet (August-September) seasons in 2016.

3.7 Container productivity:

Containers were ranked from most to least productive are tabulated in table 5. The most productive containers for *Ae. aegypti* classified by shape, use and material were plastic drums used for water storage and washing .However, those did not produce more than 16% of all immature collected (Table 5). As many as seven different classes of containers (various shape, use and material combinations) produced 72% of *Ae. aegypti*. The various categories consisted of cement and plastic tanks used for washing; mud and metal pots, buckets and mud drums used for washing; plastic and metal pots used for irrigation; glass and metal pots without use; metal and plastic pots used for pets; mud pots used for flowers; plastic drums used for irrigation; plastic drums used for drinking, plastic buckets used for irrigation, wood and plastic buckets without use, and plastic buckets used for drinking purpose.

Table 5: Most productive *Aedes aegypti* containers as classified by shape, use and material.

	Container class						
Rank	**Shape**	**Use**	**Material**	**No. positive container**	***Ae. aegypti* larvae + pupae**	**Container productivity (%)**	**Cumulative productivity (%)**
1	Drum	Washing	Plastic	21	78	16.1	16.1
2	Pot	Garbage	Plastic	25	74	15.3	31.4
3	Bucket	Washing	Plastic	14	48	9.9	41.3
4	Tire	Garbage	Rubber	10	50	10.3	51.6
5	Pot	Washing	Plastic	11	38	7.9	59.5
6	Drum	Washing	Metal	6	31	6.4	65.9
7	Drum	Dishwashing	Plastic	11	30	6.2	72.1
8	Various	Various	Various	37	135	27.9	100
Total				136	484	100	

Container productivity: Percentage of total pupae produced by each container class.

For *Ae. albopictus* the containers were ranked in the same way as for *Ae. aegypti*. Discarded plastic pots were found to be the most productive container for *Ae. Albopictus* which produced 18.4% of all immature collected. Seven different different classes of containers produced 73.4% of *Ae. albopictus*. The various category consisted of plastic tanks, metal pots metal and mud drums, metal and mud buckets used for washing;

plastic and metal pots used for irrigation; metal and glass pots without use; plastic pots used for pets; mud and plastic pots used for flowers; plastic drums, plastic buckets used for storage and drinking; cement drums used for dish washing; plastic and mud buckets use for irrigation, wood and plastic buckets without use and plastic buckets used for dish washing purpose.

Table 6: Most productive Aedes albopictus containers as classified by shape, use and material.

Rank	Shape	Use	Material	No. of positive containers	Ae. albopictuslarvae + pupae	Container productivity	Cumulative productivity
1	Pot	Garbage	Plastic	26	143	18.4	18.4
2	Bucket	Washing	Plastic	18	103	13.3	31.7
3	Drum	Washing	Plastic	22	95	12.2	43.9
4	Pot	Washing	Plastic	13	67	8.6	52.5
5	Drum	Dishwashing	Plastic	13	64	8.2	60.7
6	Pot	Garbage	Mud	8	53	6.8	67.5
7	Tire	Garbage	Rubber	9	46	5.9	73.4
8	Various	Various	Various	43	205	26.4	100
Total				152	776	100	

Container productivity: Percentage of total pupae produced by each container class.

3.8 Comparisons of mosquito density between areas and seasons:

The negative binomial regression model analysis on comparing *Ae. aegypti* (larvae + pupae), *Ae. albopictus* (larvae + pupae) and *Culex* spp. between areas in each season and between seasons are shown in table 7. Not significantly differed between residential and non-residential but significantly differed between the dry and wet seasons.

Table 7: Incidence rate ratios (IRR) (95% confidence intervals) of immature mosquitoes per container in relation to areas (for each season) and season (across areas) in 2016.

Variable	Level	*Ae. Aegypti*		*Ae. Albopictus*		*Culex* spp.
		Larvae	Pupae 1	Larvae	Pupae	
Dry season(n = 945)						
House type	Non-residential	1	-	1	1	1
	Residential	0.81 [0.16-4.13] P=0.805	-	1.12 [0.26-4.72] P=0.882	1.29 [0.10-16.73] P=0.845	0.90 [0.38-2.14] P=0.807
Wet season (n = 833)						
House type	Non-residential	1	1	1	1	1
	Residential	0.71 [0.39-1.30] P=0.274	0.67 [0.35-1.30] P=0.242	0.59 [0.30-1.18] P=0.134	0.67 [0.36-1.27] P=0.220	0.43 [0.16-1.13] P=0.087
Across house types (n=1779)						
Season	Wet season	1	1	1	1	1
	Wet season	14.48 [8.27-25.34] P<0.0001	1.16×10 into 8 [--] P=0.98	5.79 [3.34-10.05] P<0.0001	14.45 [8.03-25.98] P<0.0001	0.55 [0.30-1.00] P=0.051

[1)]No *Aedes aegypti* pupae collected in the dry season.

4. Discussion and Conclusion

The present study highlights the importance of key productive container types for dengue vectors which play pivotal role for the development to their adult stage, as these were different from the *Stegomyia* indices. This difference has been determined elsewhere previously also (Focks *et al*. 2000; Focks & Alexander 2006; Lenhart *et al*. 2006; Romero-Vivas *et al*. 2006; Troyo *et al*. 2007).

As the specific findings are detailed, the *Stegomyia* indices, Container index, House index, and Breteau index were higher in non-residential compared to residential area. The number of mosquitoes per containers was found higher in non-residential (0.60) than in the residential area (0.40). However, when testing, there were no significant differences found between areas for all species (*Ae. aegypti*, *Ae. albopictus, Culex* spp.). It means that, P value is greater than 0.05 in both dry and wet seasons for *Ae. aegypti*, *Ae. albopictus* and *Culex* spp. Non-residential areas include garbage recycling centres (1), metal workshops (2), tire repair shop (1), cement block factories (2), Offices (6), School (2), grocery shops (3), temple (2), restaurants (6), electronic shop (1), furniture factories (2), rice mill (1), and mud statue factories (3).

The reason for higher production of mosquitoes in non-residential areasin the present study may be due to more bushes in outdoor premises and locations and presence of most favourable breeding containers such as discarded plastic pots, and rubber tires fill up with fresh rainwater in repairing shop and recycling centres. The mosquito infestation was higher in residential houses compared to non-residential premises but some of the non-residential houses (recycling centres) were highly infested with *Ae. aegypti* mosquito than residential houses specially those houses which were nearby to highly infested residential houses in Rio de Janeiro, Brazil (Dos *et al*. 2010). It shows the presence of high productivity of breeding containers may be influenced by highly infested houses nearby in non-residential premises. In the present study, the density (mosquitoes/containers) was lower in the residential areas in comparison to non-residential areas. Though, overall, there were no significant differences in mosquito productivity betweenthe areas.The percentage of pupal *Ae. aegypti* production in non-residential sites in the Amazonian city of Iquitos, Peru (Morrison *et al*. 2006) and in Merida city, Mexico (Baak-Baak *et al*. 2014) when comparative studies were carried out between residential and non-residential sites,the greater number of productions of *Ae. aegypti* immature were recorded in vacant lots where there were abundant vegetation and often being located near residential premises and contained large or small size discarded water filled containers which became favourable place to breed adult mosquitoes and suitable place for the immature development compared to residential houses. Further, non-residential premises such as tire repair shops, metal workshops were infested highly with *Ae. aegypti* than residential premises (Lagrotta *et al*. 2008).

In the present study, people found to be use plastic drums, the most productive containers for *Ae. aegypti,* for washing purpose. Other containers observed were discarded plastic pots. Those were responsible for 31.3% of larvae/pupae production. Likewise, discarded plastic pots and plastic buckets used for washing furthering higher container productivity (31.7%) for *Ae. albopictus*. As many as seven different containers class (various shape, use and material combinations) only found to be produce 72-74% of all immature *Ae. aegypti* and *Ae. albopictus*. Most of the black, blue, and yellow coloured middle size plastic drums and buckets used for washing in residential houses were kept outside with lid remained open favouring oviposition for *Aedes* mosquitoes. The small size discarded plastic pots lying outdoors in non-residential areas can accumulate rainwater and favourable breeding place for dengue mosquitoes. No immature *Aedes* mosquitoes were recorded from those containers with covered lid, light and transparent coloured plastic gallons and jars, but very few numbers were collected from large sized plastic and cement tanks.

Findings of Koenraadt *et al*. (2007) showed that the most productive containers classified by shape, use, and material for pupal *Ae. aegypti* were earthen jars and cement tank used for washing purpose, which were responsible for 59% pupae production. The large sized containers with dark coloured and organic materials harbour more immature dengue mosquitoes than that of light-coloured containers (Baak-Baak *et al*. 2014).

Discarded tires, metal drums, plastic drums, and mud pots were found as the most productive container for *Ae. aegypti* and *Ae. albopictus* from Lalitpur and Kathmandu district of Nepal (Gautam *et al*. 2012). On the other hand, the findings from this study shows that the most productive containers in Lalitpur district for *Ae. aegypti* and *Ae. albopictus* were plastic pots, drums and buckets which is due to water storage practice by the communities. The variation of productive container types reflects the environmental and social settings (Jahansson *et al*. 2009).

The differences between the dry and wet seasons were noticeable while the number of larvae/pupae of *Ae. aegypti* and *Ae. albopictus* were found higher in the wet season (August and September) compared to the dry season (May and June) due to the increased temperature, humidity and rainfall favoring vector breeding in the wet season. In spite of water storage for domestic use was enhanced in the dry season, pupal productivity was found higher during the wet season. This was possibly due to the vectors' preference of those containers filled with rainwater, lying in shady places, and that were remained undisturbed.

The *Stegomyia* indices, despite being poor proxies for adult abundance, indicate the absence or presence of dengue vectors. The Container index, House index, and Breteau index were also higher in wet season compared to dry season. The statistical analysis negative binomial regression model at 95% confidence interval showed highly significant differences ($P<0.05$) betweendry season and wet seasons, indicating that population of both species were higher in wet season than in dry season. In case of *Culex* spp., mosquito population were higher in dry season than wet season ($P = 0.51$).

It means in dry season, most of the containers were dry, but after monsoon most of the containerswere filled up with fresh water which became favourable breeding place for mosquitoes. According to (Gautam *et al*. 2012; Dhimal *et al*. 2015), abundance of dengue mosquitoes follows seasonal patterns in Nepal. The larva/pupae abundance in Lalitpur and Kathmandu district were significantly higher in wet season (monsoon and post- monsoon) compared to pre-monsoon and winter season when the containers were fill up with fresh water. In this study also immature mosquito abundance was significantly higher in the wet season compared to the dry season. *Ae. Albopictus* was the most abundance species recorded from this study whichmay be due to the presence of vegetation, since *Ae. albopictus* prefer vegetation. Study conducted in Mexico shows that, the most abundance species found was *Ae. albopictus* followed by *Cx. quinquefasciatus* because of abundant vegetation (Baak-Baak *et al*. 2014). In the previous study conducted by Dhimal *et al*. in 2015 in Lalitpur district, also concluded *Ae. albopictus* as the most abundant species followed by *Ae. aegypti*. Furthermore, in this study a greater number of immature *Ae. aegypti* as well as *Ae. albopictus* had recorded from the containers lying outdoor locations rather than indoor containers which coincides to the study conducted in central Nepal (Dhimal *et al*. 2015) and India (Vijayakumar *et al*. 2014).

Overall, 2107 immature mosquitoes were collected during field survey, which includes *Ae. albopictus*, *Ae. aegypti*, *Culex* spp., *Aedes* spp., *Anopheles* spp. and other unidentified species. Among them abundance of *Ae. albopictus* from residential areas in post-monsoon season was highest followed by *Culex* spp. Abundance was high in first week of August (5^{th} field) for both *Ae. aegypti* and *Ae. albopictus*. After monsoon, most of the containers contain fresh water which become favourable place for oviposition for adult mosquitoes.

This finding concludes that the most potential breeding containers were found in non-residential areas than that of residential areas. However, mosquito abundance was low and there was not any significance difference between areas. This may be due to fewer containers found in non-residential sites. Seven different container classes (various shape, use and material combinations) only produced 72–74% of immature dengue mosquitoes, thus almost all containers searched were found productive. Containers in non-residential areas near to residential sites found positive with larvae and pupae. In non-residential houses and surrounding outdoors of the study sites contain more unused disposable plastic, metal and mud pots and discarded tires with vegetation. In such containers rainwater stored during monsoon and become favourable places to breed for mosquitoes.

Dengue fever is an emerging disease for Nepal, expanding from the lowlands to higher altitudes. It means that there could be a higher chance of risk of dengue transmission in future. Vector surveillance with larval/pupal control methods in Nepal were only focused on residential areas ignoring non-residential sites. The findings of this study suggest determination of pupal productivity would be best during the wet season that provides a vector surveillance tool for the specific container types whereby the most productive wet containers can be targeted including non-residential areas for vector management. In addition, this approach can be more cost-effective than managing or treating all containers without targeting any specific container type. However, further studies should be carried out in future to quantify the immature dengue mosquito production in residential verses non-residential areas.

Acknowledgements

The first author is grateful for the research grant from the Department of Ecology and Natural Resource Management (INA), Norwegian University of Life Sciences, Ås, Norway for providing research grant. We are thankful to the Natural History Museum,

Tribhuvan University, Swayambhu, Kathmandu, Nepal for laboratory support. We thank Sabita Oli for the field assistance.

References

Baak-Baak, C. M., R. Arana-Guardia, N. Cigarroa-Toledo, M. A. Loroño-Pino, G. ReyesSolis, C. Machain-Williams, J. B. Beaty, L. Eisen, and J. E. Garcia-Rejón, 2014. Vacant Lots: Productive Sites for *Aedes* (*Stegomyia*) *aegypti* (Diptera: Culicidae) in Mérida City, Mexico. Journal of Medical Entomology, 51(2), 475–483.
DOI: 10.1603/ME13209
PMID: 24724299 PMCID: PMC4064362

Bhatt, S., P. W. Gething, O. J. Brady, J. P. Messina, A. W. Farlow, C. L. Moyes, J. M. Drake, J. S. Brownstein, A. G. Hoen, O. Sankoh, D. B. George, T. Jaenisch, G. R. W. Wint, C. P. Simmons, T. W. Scott, J. J. Farrar, S. I. Hay, and M. F. Myers, 2013. The global distribution and burden of dengue. Nature, 496(7446), 504-507.
DOI: 10.1038/nature12060
PMID:23563266 PMCID:PMC3651993

Bonizzoni, M., G. Gasperi, X. Chen, and A. A. James, 2013. The invasive mosquito species *Aedesalbopictus:* current knowledge and future perspectives. Trends in Parasitology, 29(9), 460-468.

Brady, O. J., and S. I. Hay, 2020. The global expansion of dengue: How *Aedes aegypti* mosquitoes enabled the first pandemic arbovirus. Annu. Rev. Entomol. 65, 191–208.
DOI: 10.1146/annurev-ento-011019-024918
PMID:31594415

Braks, M. A., N. A. Honório, R. Lourenço-De-Oliveira, S. A. Juliano, and L. P. Lounibos, 2003. Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in southeastern Brazil and Florida. Journal of Medical Entomology, 40(6), 785– 794.
DOI:10.1603/0022-2585-40.6.785
PMID:14765654

Braks, M. A. H., N. A. Honório, L. P. Lounibos, R. Lourenço-de-Oliveira, and S. A. Juliano, 2004. Interspecific competition between two invasive species of container mosquitoes, Aedes aegypti and Aedes albopictus (Diptera: Culicidae), in Brazil. Annals of the Entomological Society of America, 97(1), 130-139.
DOI:10.1603/0013-8746(2004)097[0130:ICBTIS]2.0.CO;2

Chen, C. D., H. L. Lee, K. W. Lau, A. G. Abdullah, S. B. Tan, and I. Sa'diyah, 2014. Biting behavior of Malaysian mosquitoes, *Aedes albopictus* Skuse, *Armigeres kesseli* Ramalingam, *Culex quinquefasciatus* Say, and *Culex vishnui* Theobald obtained from urban residential areas in Kuala Lumpur. Asian Biomed., 8(3), 315–21.
DOI: 10.5372/1905-7415.0803.295

Christophers, R., 1960. *Aedes aegypti* (L.) the yellow fever mosquito: its life history, bionomics and structure. The Syndics of the Cambridge University Press, London, UK 721pp.

Darsie, Jr. R. F. and S. P. Pradhan, 1990. The mosquitoes of Nepal: Their identification, distribution and biology. Mosquito Systematics, 22(2), 69-130.

Deng, S. Q., X. Yang, Y. Wei, J. T. Chen, X. J. Wang, and H. J. Peng, 2020. A review on dengue vaccine development. Vaccines, *8*(1), 63.
DOI: 10.3390/vaccines8010063
PMID:32024238 PMCID:PMC7159032

Dhimal, M., I. Gautam, H. D. Joshi, R. B. O'Hara, B. Ahrens, and U. Kuch, 2015. Risk factors for the presence of chikungunya and dengue vectors (*Aedes aegypti* and *Aedes albopictus*), their altitudinal distribution and climatic determinants of their abundance in central Nepal. PloS Neglected Tropical Disease, 16; 9(3):e0003545. doi: 10.1371/journal.pntd.0003545.
DOI: 10.1371/journal.pntd.0003545
PMID:25774518 PMCID:PMC4361564

Dos Reis, I. C., N. A. Honório, C. T. Codeço, M. D. A. F. M. Magalhães, R. Lourenço-deOliveira, and C. Barcellos, 2010. Relevance of differentiating between residential and non-residential premises for surveillance and control of *Aedes aegypti* in Rio de Janeiro, Brazil. Actatropica, 114(1), 37–43.
DOI: 10.1016/j.actatropica.2010.01.001
PMID:20074538

Fenemore, P. G. 2006. Applied entomology. New Age International, India. 292 pp.

Focks, D., and N. Alexander, 2006. Multicountry study of *Aedes aegypti* pupal productivity survey methodology: findings and recommendations. World Health Organization (TDR/IDE/Den/06.1)
DOI: 10.4269/ajtmh.2000.62.11
PMID:10761719

Gautam, I., M. N. Dhimal, S. R. Shrestha, and A. S. Tamrakar, 2009. First Record of *Aedes aegypti* (L.) Vector of Dengue Virus from Kathmandu, Nepal. Journal of Natural History Museum, 24:156 –164. DOI: 10.3126/jnhm.v24i1.2298

Gautam, I., A. K. C, R. Tuladhar, B. D. Pandey, A. S. Tamrakar, R. Byanju, M. Dhimal, K. Aryal, and U. Kuch, 2012. Container preference of the Asian Tiger Mosquito (*Aedes albopictus*) in Kathmandu and Lalitpur districts of Nepal. Journal of Natural History Museum, 26, 181–193. DOI: 10.3126/jnhm.v26i0.14142

Gratz, N. G., 2004. Critical review of the vector status of *Aedes albopictus*. Medical and Veterinary Entomology, 18(3), 215–227. DOI: 10.1111/j.0269-283X.2004.00513.x PMID:15347388

Gubler, D. J., 1998. Resurgent vector-borne diseases as a global health problem. Emerging Infectious Diseases, 4(3), 442–450. DOI: 10.3201/eid0403.980326 PMID:9716967 PMCID:PMC2640300

Gubler, D. J., 2002. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends in Microbiology, 10(2), 100–103. DOI: 10.1016/S0966-842X(01)02288-0 PMID:11827812

Gubler, D. J., 2006. Dengue/dengue haemorrhagic fever: history and current status. In Novartis foundation symposium, Vol. 277, p3. DOI: 10.1002/0470058005.ch2 PMID:17319151

Hadinegoro, S. R. S., 2012. The revised WHO dengue case classification: does the system need to be modified? Paediatrics and International Child Health, 32(1), 33-38. DOI: 10.1179/2046904712Z.00000000052 PMID:22668448 PMCID:PMC3381438

Hawley, W. A., 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 1, 1–39.

Ho, B. C., Y. C. Chan, and K. L. Chan, 1973. Field and laboratory observations on landing and biting periodicities of *Aedes albopictus* (Skuse). The Southeast Asian Journal of Tropical Medicine and Public Health, 4(2), 238.

Honório, N. A., C. T. Codeço, F. C. Alves, M. D. A. Magalhães, and R. Lourenço-de-Oliveira, 2009. Temporal distribution of *Aedes aegypti* in different districts of Rio de Janeiro, Brazil, measured by two types of traps. Journal of Medical Entomology, 46(5), 1001–1014. DOI: 10.1603/033.046.0505 PMID:19769029

Jacobs, M., 2000. Dengue: emergence as a global public health problem and prospects for control. Transactions of the Royal Society of Tropical Medicine and Hygiene, 94(1), 7-8. DOI: 10.1016/S0035-9203(00)90416-4 PMID:10748886

Johansson M. A., F. Dominici, and G. E. Glass, 2009. Local and GlobalEffects of Climate on Dengue Transmission in Puerto Rico. PLoSNegl Trop Dis 3(2): e382. DOI:10.1371/journal.pntd.0000382 PMID:19221592 PMCID:PMC2637540

Koenraadt, C. J. M., J. W. Jones, R. Sithiprasasna, and T. W. Scott, 2007. Standardizing container classification for immature *Aedes aegypti* surveillance in KamphaengPhet, Thailand. Journal of Medical Entomology,44(6), 938–944. DOI: 10.1093/jmedent/44.6.938 PMID:18047191

Kolimenakis, A., S. Heinz, M. L. Wilson, V. Winkler, L. Yakob, A. Michaelakis, D. Papachristos, C. Richardson, and O. Horstick, 2021. The role of urbanisation in the spread of Aedes mosquitoes and the diseases they transmit-A systematic review. PLoS Negl Trop Dis. 2021 Sep 9;15(9):e0009631. DOI: 10.1371/journal.pntd.0009631 PMID:34499653 PMCID:PMC8428665

Kroeger, A., A. Lenhart, M. Ochoa, E. Villegas, M. Levy, N. Alexander, and P. J. McCall, 2006. Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: cluster randomised trials. Bmj, 332(7552), 1247- 1252. DOI: 10.1136/bmj.332.7552.1247 PMID:16735334 PMCID:PMC1471956

Lagrotta, M. T. F., W. D. C. Silva, R. Souza-Santos, 2008. Identification of key areas for Aedes aegypti control through geoprocessing in Nova Iguaçu, Rio de Janeiro State, Brazil. Cadernos de Saúde Pública, *24*, 70-80. DOI: 10.1590/S0102-311X2008000100007 PMID:18209835

Lambrechts, L., T. W. Scott, and D. J. Gubler, 2010.

Consequences of the expanding global distribution of *Aedes albopictus* for dengue virus transmission. PLOS Neglected Tropical Diseases, 4(5), 646. DOI:10.1371/journal.pntd.0000646 PMID:20520794 PMCID:PMC2876112

Lenhart A. E., C. E. Castillo, M. Oviedo, and E. Villegas, 2006. Use of the pupal-demographic survey technique to identify the epidemiologically important types of containers producing *Aedes aegypti* in a dengue-epidemic area of Venezuela. Ann Trop Med Parasitol. 100 Suppl 1:S53–S59. DOI: 10.1179/136485906X105516 PMID:16630391

Malla, S., G. D. Thakur, K. S. Shrestha, K. M. Banjeree, B. L. Thapa, G. Gongal, P. Ghimire, P. B. Upadhyay, P. Gautam, S. Khanal, A. Nisak, G. R. Jarman, and V. R. Gibbons, 2008. Identification of all dengue serotypes in Nepal. Emerging Infectious Diseases, 14 (10). DOI: 10.3201/eid1410.080432 PMID:18826846 PMCID:PMC2609890

Messina, J. P., O. J. Brady, N. Golding, M. U. Kraemer, G.W. Wint, and S. E. Ray, 2019. The current and future global distribution and population at risk of dengue. Nature Microbiology 2019.1. PMID: 30546101.

Morrison, A. C., M. Sihuincha, J. D. Stancil, E. Zamora, H. Astete, J. G. Olson, C. OreVidal, and T. W. Scott, 2006. *Aedes aegypti* (Diptera: Culicidae) production from nonresidential sites in the Amazonian city of Iquitos, Peru. Annals of Tropical Medicine & Parasitology, 100(Suppl1) 1:73-86. DOI: 10.1179/136485906X105534 PMID:16630393

Muir, L. E., and B. H. Kay, 1998. *Aedes aegypti* survival and dispersal estimated by markrelease-recapture in northern Australia. The American Journal of Tropical Medicine and Hygiene, 58(3), 277–282. DOI: 10.4269/ajtmh.1998.58.277 PMID:9546403

Nathan, M. B., D. A. Focks, and A. Kroeger, 2006. Pupal/demographic surveys to inform dengue-vector control. Annals of Tropical Medicine & Parasitology, 100 Suppl 1:S1-S3. DOI: 10.1179/136485906X105462 PMID:16630386

Pandey, B. D., S. K. Rai, K. Morita, and I. Kurane, 2004. First case of Dengue virus infection in Nepal. Nepal Medical College Journal: NMCJ, 6(2), 157-159.

Pandey, B. D., K. Morita, T. T. Khanal, I. Miyazaki, T. Ogawa, S. Inoue, and I. Kurane, 2008. Dengue virus, Nepal. Emerging Infectious Diseases, 14(3): 514-5. DOI: 10.3201/eid1403.070473 PMID:18325280 PMCID:PMC2570825

Pandey, B. D., T. Nabeshima, K. Pandey, S. P. Rajendra, Y. Shah, B. R. Adhikari, G. Gupta, I. Gautam, M. M. Tun, R. Uchida, and M. Shrestha, 2013. First isolation of dengue virus from the 2010 epidemic in Nepal. Tropical Medicine and Health, 41(3), 103-111. DOI: 10.2149/tmh.2012-17 PMID:24155651 PMCID:PMC3801155

Pasteur, S., 2016. Dengvaxia®, World's First Dengue Vaccine, Approved in Mexico.

Paupy, C., H. Delatte, L. Bagny, V. Corbel, and D. Fontenille, 2009. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. Microb. Infect. 11, 1177–1185. DOI: 10.1016/j.micinf.2009.05.005 PMID:19450706

Peters, W., and S. C. Dewar, 1956. A preliminary record of the megarhine and culicine mosquitoes of Nepal with notes on their taxonomy (Diptera: Culicidae). Indian Journal of Malariology, 10(1), 37-51.

Petrić, D., R. Bellini, E. J. Scholte, L. M. Rakotoarivony, and F. Schaffner, 2014. Monitoring population and environmental parameters of invasive mosquito species in Europe. Parasites & Vectors, 7(1), 1-14. DOI: 10.1186/1756-3305-7-187 PMID:24739334 PMCID:PMC4005621

Ponlawat, A., and L. C. Harrington, 2005. Blood feeding patterns of *Aedesaegypti* and *Aedesalbopictus*in Thailand. Journal of Medical Entomology, 42(5), 844–849. DOI: 10.1093/jmedent/42.5.844 PMID:16363170

Ponlawat, A., J. G. Scott, and L. C. Harrington, 2005. Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. Journal of Medical Entomology, 42(5), 821– 825. DOI: 10.1093/jmedent/42.5.821 PMID:16363166

Pun, S. B., 2011. Dengue-an emerging disease in Nepal. Journal of Nepal Medical Association, 51(184), 203-208.
DOI: 10.31729/jnma.33

Romero-Vivas CME., P. Padilla, and AKI Falconar, 2006. Pupal-productivity surveys to identify the key container habitats of *Aedes aegypti* (L.) in Barranquilla, the principal seaport of Colombia. Annals of Tropical Medicine and Parasitology 100 Suppl, Issue: S87–S95.
DOI:10.1179/136485906X105543
PMID:16630394

Rueda, L. M., 2004. Pictorial keys for identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. Zootaxa (598). Magnolia Press, Aukland, Newzealand.
DOI: 10.11646/zootaxa.589.1.1

Sota, T., and M. Mogi, 1992. Interspecific variation in desiccation survival time of *Aedes* (*Stegomyia*) mosquito eggs is correlated with habitat and egg size. Oecologia, 90(3), 353– 358.
DOI: 10.1007/BF00317691
PMID:28313521

Su, C. I., C. H. Tseng, C. Y. Yu, and M. M. Lai, 2016. SUMO modification stabilizes dengue virus nonstructural protein 5 to support virus replication. Journal of Virology, 90(9), 4308-4319.
DOI: 10.1128/JVI.00223-16
PMID:26889037 PMCID:PMC4836324

Tauil, P. L., 2001. Urbanization and dengue ecology. Cadernos de saúdepública, 17, 99-102.
DOI: 10.1590/S0102-311X2001000700018
PMID:11426270

Troyo, A., DO. Fuller, O. Calderon-Arguedas, and J. C. Beier, 2007. A geographical sampling method for surveys of mosquito larvae in an Urban area using high-resolution satellite imagery. J. Vector Ecology. 33 (1): 1-7. DOI: 10.3376/1081-1710(2008)33[1:AGSMFS]2.0.CO;2
PMID:18697301

Vijayakumar, K., T. S. Kumar, Z. T. Nujum, F. Umarul, and A. Kuriakose, 2014. A study on container breeding mosquitoes with special reference to *Aedes* (*Stegomyia*) *aegypti* and *Aedes albopictus* in Thiruvananthapuram district, India. Journal of Vector Borne Diseases, 51(1), 27–32
DOI: 10.4103/0972-9062.130145
PMID:24717199

Vikram, K., B. N. Nagpal, V. Pande, A. Srivastava, R. Saxena, A. Anvikar, and R. Paul, 2016. An epidemiological study of dengue in Delhi, India. Actatropica, 153, 21-27.
DOI: 10.1016/j.actatropica.2015.09.025
PMID:26433076

Weaver, S. C., and N. L. Forrester, 2015. Chikungunya: evolutionary history and recent epidemic spread. Antivir. Res. 120, 32-39.
DOI: 10.1016/j.antiviral.2015.04.016
PMID:25979669

World Health Organization, 2006. Outbreak investigation of DF in Nepal, SEARO availablefromhttp://www.searo.who.int/LinkFiles/Dengue_dengue_Nepal.pdf.

World Health Organization, 2008. Dengue haemorrhagic fever. Factsheet No 117, revised May 2008. Geneva. World Health Organization, 2, 25-28.

World Health Organization, 2009. Epidemic, and Pandemic Alert. Dengue: guidelines for diagnosis, treatment, prevention and control.

Yang, H., M. D. L. D. G. Macoris, K. C. Galvani, M. T. M. Andrighetti, and D. M. V. Wanderley, 2009. Assessing the effects of temperature on the population of *Aedes aegypti*, the vector of dengue. Epidemiology & Infection, 137(8), 1188–1202.
DOI: 10.1017/S0950268809002040
PMID:19192322

Factors Associated with the use of Institutional Delivery Services by Mothers in a Community of Gorkha, Nepal

Archana Bagale[1*] and Shristi Lamichhane[2]

[1]Assistant Professor, Patan Academy of Health Science, School of Nursing and Midwifery
[2]Registered Nurse, Nepal

***CORRESPONDING AUTHOR:**

Archana Bagale
Email:archanamachhindra2045@gmail.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI : https://doi.org/10.3126/njst.v21i2.67157

Date of Submission: Feb 5, 2023

Date of Acceptance: Mar 3, 2024

ABSTRACT

Institutional delivery is a delivery that takes place at any medical facility staffed by skilled delivery assistance. A descriptive cross-sectional study design was adopted to find out the factors associated with institutional delivery service utilization among mothers. The study was done in wards 6 and 11 of Gorkha Municipality in Gorkha district. The sample size was 80. The data were analysed using SPSS 16. The majority of the respondents, i.e., 97.5%, were literate, and 60% of the respondents were homemakers. 38.5% of the respondent's husband was involved in foreign employment. Obstetric factors for institutional delivery depict nearly three-fourths of the total respondents, i.e., 72.5% of mothers marry at the age of 15-20 years; nearly half, i.e., 48.8% of mothers deliver babies at the age of 21–25; and the majority of the respondents, i.e., 97.5%, visited for an ANC checkup more than four times. Only 18.8% had complications during pregnancy. The majority of the respondents, i.e., 97.5%, thought health institutions were the best place for delivery, of which 60.3% chose health institutions for quality services. The majority of the respondents, 93.8%, delivered babies at health institutions. Enabling factors for institutional delivery depict nearly two-thirds of the respondents, i.e., 62.5%, making their own decisions. The majority of the respondents, i.e., 85.7%, received delivery services by a nurse; nearly half of the respondents, i.e., 47.5%, travelled 30–60 minutes. More than half of the respondents (55.0%) went to health institutions by vehicle. Similarly, 57.1% of the respondents received health care within 15 minutes. The present study concluded that, in spite of the higher proportion of institutional delivery, no significant association was found among the selected socio-demographic variables, i.e., educational status, spouse educational status, ethnicity, religion level, and monthly income.

Key words: Delivery, Factors, Institution, Service

1. Introduction

Institutional delivery service utilization is one of the most important factors in reducing maternal death, and the proportion of women who delivered with the assistance of a skilled birth attendant is one of the indicators in every country's health plans. Maternal death is highest among countries with less skilled professionals, such as a trained midwife, nurse, doctor, or other trained health professionals (Tiruye *et al*. 2018). It has been noted that over half a million maternal deaths occur every year, 99% in developing countries (86% in sub-Saharan Africa and Asia), and for each of these deaths, an estimated further 30 women will become disabled, injured, or ill owing to pregnancy. Complications of pregnancy and childbirth are taking away the lives of an estimated 303,000 women annually worldwide (Teklehaymanot *et al*. 2016). Although institutional delivery service utilization ensures safe birth and is a key to reducing maternal mortality, interventions in the community and/or institutions were unsatisfactory in reducing maternal mortality. Institutional delivery service utilization is affected by the interaction of personal, socio-cultural, behavioral, and institutional factors **(**Wako & Kassa 2017).

In Nepal, a high proportion of maternal deaths is most likely due to the fact that over 63% of births take place at home. Evidence suggests that appropriate delivery care and skilled attendance at birth ensure safe delivery and help identify the onset of complications in time. In-depth understanding of the factors associated with the utilization of institutional delivery services is of paramount importance to unleashing the barriers to promoting the utilization of institutional delivery (Thapa *et al*. 2019). Globally, there were an estimated 289,000 maternal deaths in 2013, yielding a maternal mortality rate (MMR) of 210 maternal deaths per 100,000 live births. Developing countries account for 99% (286,000) of the global maternal deaths; Sub-Saharan Africa accounts for 50% of all maternal deaths worldwide; and South Asia accounts for 35%, which is in extreme contrast with the high-income countries. Despite the commitment of the international community to reduce MM, the magnitude of the problem remains immense. Thus, the goal of MDG 5, i.e., reducing the MM by three quarters by the year 2015, has barely been achieved. Haemorrhage and hypertensive disorders are the leading causes of maternal mortality in developing countries (Amentie *et al*. 2017). An international conference on population and development aims to have at least 90% of deliveries attended by skilled health care providers by 2015 as a strategy for reducing maternal mortality. However, in developed countries, 94% of births are attended by health care personnel. Whereas in developing countries like Nepal, only 59% of deliveries are attended by institutions (NDHS 2017). The maternal mortality rate in Nepal was found to be 258 per 100,000 live births (2017). Maternal mortality due to unsafe abortion, prolonged labour, eclampsia, and other reasons has been a major problem across the globe, especially in developing countries. Most of the deliveries occur outside of health care facilities and are assisted by nonprofessionals (NDHS 2017).

From the above statement, the trend of institution delivery seems to be increasing, so the aim is to generate the best evidence on the determinants of institutional delivery service utilization and to find out the associated factors related to institutional delivery in the Gorkha community. Likewise, such a study has not been conducted in this community, so I chose this topic as my research topic.

2. Research Methodology

Study Design: Descriptive cross-sectional study design.

Study Area and Study Population: The study was conducted in Gorkha Municipality, wards 6 and 11, of Gorkha district, which is situated in the western part of Nepal.

Sampling Technique: Non-probability, Purposive Sampling Technique

Sampling Unit: The sampling unit was the mother having a baby less than 5 years of age.

Sample Size: 80

Method for Data Collection: Face-to-face interview method was used as a method of data collection for this study design.

Tools for Data Collection: A semi-structured tool was used for data collection for the study.

Ethical Consideration

Ethical clearance was taken from IRB of Yeti Health Science Academy, Maharajgunj, Kathmandu.

Verbal and written consent was obtained from the respondents before data collection. Confidentiality and anonymity were maintained throughout the study.

The purpose and methods were clearly informed. The obtained information was used only for the necessary study purpose.

3. Results

Table 1 depicts that 8.8% of pregnant mothers were of teen age. Near to half percentage of respondent i.e. 43.8% of the respondents were between the ages of 26-30 years. Majority of the respondents i.e. 97.5% were literate, and only 2.5% of respondents were illiterate. Among literate respondents, (n=78), 41.02% were of primary level whereas least of respondents 6.42% were of university level. Similarly, spouse educational status wise, majority of the respondents 96.25% were literate and only 3.75% were illiterate. Among literate respondents (n=77) about one third of the respondents i.e. 33.7% were of lower secondary level, and 5.19% were of university level. Occupation wise, 60% of the respondents were homemaker and least of the respondent only 1.3% were labor.

Table1: Socio demographic information of respondents (n=80)

Variables	Frequency(Percentages)
Age group	
15-20	7(8.8%)
21-25	22(27.5%)
26-30	35(43.8%)
31-35	16(19.9%)
Ethnicity	
Janajati	35(43.75%)
Brahmin/ Chhetri	24(30%)
Dalit	17(21.25%)
Muslim	4(5.00%)
Religion	
Hindu	69(86.25%)
Christian	5(6.25%)
Muslim	4(5%)
Buddhist	2(2.5%)
Educational Status	
Literate	78(97.5%)
Illiterate	2(2.5%)
Women Education (n=78)	
Lower Secondary	32(41.02%)
Secondary	30(38.46%)
Higher Secondary	11(14.10%)
University	5(6.41%)
Spouse Educational Status	
Literate	77(96.25%)
Illiterate	3(3.75%)
Spouse Education (n=77)	
Lower Secondary	26(33.7%)
Secondary	25(32.46%)
Higher Secondary	22(28.57%)
University	4(5.19%)
Women Occupation	
Homemaker	48(60%)
Sales/Service	15(18.75%)
Agriculture	13(16.25%)
Technical/Professional	3(3.75%)
Labor	1(1.25%)
Spouse Occupation	
Abroad	28(35%
Sales/Service	22(27.5%)
Technical/Professional	14(17.5%)
Agriculture	9(11.3%)
Labor	7(8.75%)

Table 2 depicts obstetric factors for institutional delivery. Among the total respondents nearly three fourth i.e. 72.5% of mother marriage at the age of 15-20 years, whereas least of the respondents 3.8% marriage at the age of 26-30. Nearly half i.e. 48.8% of mother deliver baby at the age of 21-25, whereas least of the respondents 10.0% delivered at the age of 26-30. Similarly, 45.0% of the respondents had 1-2 numbers of children, whereas only 2.5% had 4 numbers of children. Majority of the respondents i.e. 97.5% visited for ANC checkup for more than 4 times, whereas least of the respondents 2.5% not visited for ANC checkup.

Table 2: Factors associated with Institutional Delivery (n=80)

Variables	Frequency(Percentage)
Age at marriage	
15-20	58(72.50%)
21-25	19(23.80%)
26-30	3(3.75%)
Age at first delivery	
15-20	33(41.25%)
21-25	39(48.75%)
26-30	8(10%)
No. of Children	
1	36(45%)
2	36(45%)
3	6(7.5%)
4	2(2.5%)
ANC Visit, n=78	
More then 4	78(100%)
Complication during Pregnancy	
Yes	15(81.3%)
Complication type (n=15)	
Post date	5(33.3%)
GHTN	5(33.3%)
Breech Presentation	2(13.3%)
Allergy	1(6.7%)
Oligohydraminous	1(6.7%)
Rh incompatibility	1(6.7%)

Table 3 depicts obstetric factors for institutional delivery. Majority of the respondents i.e. 97.5% thought health institution the best place for delivery, whereas least of the respondents only 2.5% thought home is the best place for delivery, in which 60.3% choose health institution for quality services. Majority of the respondents, 93.8% delivered baby at health institution whereas only 3.8% delivered baby at home. Likewise, 62.3% of the family member suggest for institutional delivery whereas, 2% were suggested by others.

Table 3: Obstetric Factors for Institutional delivery (n=80)

Variables	Frequency (Percentage)
Best place for Delivery	
Institution	78(97.5%)
Home	2(2.5%)
Reason for Home Delivery (n=2)	
No response	2(100%)
Reason for Institution Delivery (n=78)	
To get quality services	47(60.30%)
Complications can be prevented easily	15(19.20%)
For safe Delivery	13(16.70%)
Accessible and Available services	3(3.80%)
Place of Delivery	
Health Institution	75(93.75%)
Home	3(3.75%)
On the way to hospital	2(2.50%)
Suggesting person for institutional delivery (n=77)	
Family	48(62.30%)
Self	24(31.20%)
Husband	3(3.90%)
Others	2(2.60%)

Among the total respondents, nearly two third i.e. 62.5% of the respondents make their decision by own, whereas 17.5% of respondents husbands used to make decision related to place of delivery. Similarly, majority of the respondent i.e. 85.7% received delivery services by nurse, whereas only 1.3% of respondent received delivery service by ANM. Likewise, in distance between home and institution nearly half i.e. 47.5% of the respondents have 30-60 minutes, whereas, only 1.3% have 1 hours distance between home and health institution. More than half of the respondents 55.0% went to health institution by vehicles whereas, 45% went by foot. And, none of the respondents prefer institution for incentive facilities. Similarly, 57.1% of the respondents received health care within 15 minutes, whereas least of the respondent i.e. only 1.3% received care after more than 1 hour. Similarly, all of the respondents prefer health institution as they were satisfied towards available

health care facilities and towards the service provider's behavior.

Table 4: Enabling Factors for Institutional Delivery (n=80)

Variables	Frequency (Percentage)
Decision making person	
Self	50(62.50%)
Mother in Law/Father in Law	16(20%)
Husband	14(17.50%)
Service provider at health Institution (n=77)	
Nurse	66(85.70%)
Doctor	10(13%)
ANM	1(1.30%)
Distance between Home and Institution	
Less than 30 minute	37(46.25%)
30-60 minutes	38(47.50%)
1-2 hours	1(1.30%)
More than 2 hours	4(4%)
ways to go health Institution	
By Vehicles	44(55%)
By foot	36(45%)
Prefer Institution for incentive facility (n=77)	
No	77(96.25%)
Service provided within after reaching Institution (n=77)	
within 15 minutes	44(57.14%)
15-30 minutes	29(37.66%)
1 hours	3(3.89%)
more than 1 hours	1(1.29%)
Satisfied towards service (n=77)	77(100%)
Satisfaction toward service providers behavior (n=77)	77(100%)

There was no significant association between delivery place and selected socio-demographic variables.

Table 5: Association between Delivery place and Socio-demographic variables (n= 80)

Variables		Delivery Place		P value
		Home	Institution	
Educational status	Literate	2	76	0.074
	Illiterate	1	1	
Spouse Educational Status	Literate	2	75	
	Illiterate	1	2	0.110
Ethnicity Level	Ungrouped caste	1	44	
	Janajati	2	33	0.578
Religion Level	Hindu	2	67	
	Others	1	10	0.362
Monthly income	1000-50000	2	69	
	51000-100000	1	8	0.304

Fisher's exact test

4. Discussion

In this study, the majority of the respondents (97.5%) were literate. Occupation-wise, 60% of the respondents were homemakers, and spouse-occupation-wise, all were employed, and 47.5% of the family had 21000–40000 income.

The finding is consistent with the similar study conducted in Kavre district; the data revealed that the majority (85.9%) were literate, whereas, spouse-wise, the majority (95.9%) were literate. The finding is not consistent among spouse occupations, as only more than half (55.3%) of respondents husbands were employed; the rest are unemployed (Shrestha & Shrestha 2017).

The finding is consistent with the similar study conducted in Palungtar, Gorkha district, as 80.5% of the mothers have attended secondary and above grades, while 85.6% of mothers reported that their husbands have attended secondary and above grades. The majority (57.2%) of the mothers were engaged in agriculture. Nearly half (49.4%) of the mothers reported that their husbands were working abroad. 40.6% of the mothers reported their monthly family income above Rs. 30,000 (Yadav & Jena 2020).

This finding is consistent with a similar study conducted in Ethiopia, where mothers who attended primary school and were above the primary educational level were almost five times more likely to give birth at a health institution than those who were uneducated. The chance of delivering in health facilities among those women whose husbands attended primary and above primary educational levels was 4.4-fold higher than that of those women whose husbands did not attend any educational level (Teklehaymanot *et al*. 2016).

Obstetric factors: In this study, about three-fourths of 72.5% of respondents had marriage at the age of 15-20 years, and 48.8% of mothers delivered babies at the age of 21–25. Likewise, the majority of the respondents, i.e., 97.5%, visited for ANC checkups more than four times, whereas the least number of respondents (2.5% not visit for ANC checkups. Likewise, 62.3% of the family members suggested institutional delivery, whereas 2% were suggested by others. The data revealed that the majority, 93.8%, had utilised health facilities for delivery, while only 3.8% had delivered at home and the rest, 2.5%, delivered on the way to the hospital. Likewise, 81.3% did not have any complications, whereas only 18.8% had complications during pregnancy. The finding is consistent with a similar study done in the Chitwan district of Nepal. The mean age at first pregnancy of respondents was 21–26 years. But the finding is not consistent among ANC visits. 74.4% of the respondents had visited the ANC clinic four or more times. 78.3% of the respondents had delivered their last child in a health care facility, while 21.7% had home delivery (Thapa *et al*. 2019).

This finding is consistent with the similar study conducted in Palungtar, Gorkha district; the study revealed that 68.3% of respondents got married at the age of ≤19 years. In total, 39.4% of the mothers had their first pregnancy at an early age (≤19 years). All the mothers reported having an antenatal care (ANC) visit during pregnancy. The prevalence of institutional and home delivery was 93.3% and 6.7%, respectively. However, the finding contradicts the results reported by the Nepal Demography and Health Survey (NDHS), which depict that only 59% of births took place in health facilities (Yadav *et al*. 2016).The finding is consistent with the study done in Kavre district, among the total participants. Almost 96.5% of them had visited for an ANC checkup. The majority (90%) of them had institutional delivery (Shrestha & Shrestha 2017).

Enabling factors: In this study, more than half (62.5%) of the respondents make their own decisions related to the place of delivery. Similarly, the majority of the respondents (85.7% received delivery services from nurses. Likewise, in terms of distance between home and institution, 47.5% of the respondents have 30–60 minutes, whereas 1.3% have 1 hour, and more than half of the respondents, 55.0%, went to health institutions by vehicle, whereas 45% went by foot. Likewise, more than half (57.1%) of the respondents received health care within 15 minutes, whereas the least (1.3%) received care after more than 1 hour. Similarly, all of the respondents preferred health institutions, as they were satisfied with the available health care facilities and the service provider's behavior.

Association of place of delivery with selected socio-demographic variables: In the current study, any of these variables—educational status of respondents, spouse's educational status, ethnicity, religion, and monthly income—are not significantly associated with the place of delivery.

The Chi-square test was done to find an association between the variables in Palungtar, Gorkha. It showed

that ethnicity, educational level of the respondent, respondents' husband's education, and monthly income were not significantly associated, whereas age at marriage, age at first pregnancy, knowledge of delivery incentives and maternal health before the recent delivery, time to receive health services at a health institution, and mothers knowledge of the differences between home and institutional delivery were statistically significant with the utilization of institutional delivery services (Yadav & Jena 2020).

5. Conclusion

The present study concluded that, in spite of the higher proportion of institutional delivery, no significant association was found among the selected socio-demographic variables, i.e., educational status, spouse educational status, ethnicity, religion level, and monthly income. But the study indicates that respondents having higher education, spouse's educational status, monthly income, age at marriage, ANC visit, associated complications, self-autonomy, availability and accessibility, and satisfaction with the services and the service provider's behaviours seem to be associated with the place of delivery.

Acknowledgements

We take this opportunity to extend our sincere thanks and indebtedness to all those people and dignitaries who helped us complete this study. Our deep appreciation goes to respondents during data collection. We would also like to express our gratitude to all those respondents who were included in this study for their kind cooperation and willingness to help us in the research work.

References

Amentie, M., M. Abdulahi, and Assosa, 2017. Utilisation of Institutional Delivery Care Services and Influencing Factors among Women of Child-Bearing Age in Assosa District, Benishangul Gumuz Regional State, West Ethiopia. Bibliography -019-0820-8.

Nepal Demographic and Health Survey 2016–2017 GHDx. (1970, January 1). Retrieved February 10, 2023, from https://ghdx.healthdata.org/record/nepal-demographic-and-health-survey-2016-2017.

Nigussie Teklehaymanot, A., A. Kebede, and K. Hassen, 2016. Factors associated with institutional delivery service utilisation in Ethiopia. *International Journal of Women's Health*, Volume 8, 463–475. https://doi.org/10.2147/ijwh.s109498.
DOI: 10.2147/IJWH.S109498
PMID:27672342 PMCID:PMC5026219

Shrestha, S., and G. K. Shrestha, 2017. Institutional delivery and its associated factors: a community-based cross-sectional study in Kavre district. Journal of Kathmandu Medical College, Vol. 6, No. 1, Issue 19. DOI: 10.3126/jkmc.v6i1.18582

Thapa, P., A. H. Bangura, I. Nirola, D. Citrin, B. Belbase, B. Bogati, B. K. Nirmala, S. Khadka, L. Kunwar, S. Halliday, N. Choudhury, A. Ozonoff, J. Tenpa, R. Schwarz, M. Adhikari, S. P. Kalaunee, S. Rising, D. Maru, and S. Maru, 2019. The power of peers: An effectiveness evaluation of a cluster-controlled trial of group antenatal care in rural Nepal. *Reproductive Health*, *16*(1). https://doi.org/10.1186/s12978-019-0820-8.
DOI: 10.1186/s12978-019-0820-8
PMID:31640770 PMCID:PMC6805428

Tiruye, G., F. Mesfin, and B. Geda, 2018. Breastfeeding technique and associated factors among breastfeeding mothers in Harar city, Eastern Ethiopia. Int Breastfeed J 13, 5.
DOI: 10.1186/s13006-018-0147-z
PMID:29434650 PMCID:PMC5791732

Wako, W.G., and D. H. Kassa, 2017. Institutional delivery service utilisation and associated factors among women of reproductive age in the mobile pastoral community of the Liban District in Guji Zone, Oromia, Southern Ethiopia: a cross-sectional study. BMC Pregnancy Childbirth. May 15;17(1):144.
DOI: 10.1186/s12884-017-1325-5
PMID:28506226 PMCID:PMC5433167

Yadav, A. K., and P. K. Jena, 2020. "Maternal health outcomes of socially marginalised groups in India,» *International Journal of Health Care Quality Assurance*, Vol. 33, No. 2, pp. 172-188. https://doi.org/10.1108/IJHCQA-08-2018-0212.
DOI: 10.1108/IJHCQA-08-2018-0212
PMID:32129579

Molecular identification of Chinese citrus fly, *Bactrocera minax* (Diptera: Tephritidae) in Nepal

Debraj Adhikari[1,2*], Resham B Thapa[1], Samudra L Joshi[3], Jason J Du[4], Roji Raut[5], Prajwal Manandhar[5], Pragun Rajbhandari[5] and Dibesh Karmacharya[5,6]

[1]Agriculture and Forestry University, Rampur, Chitwan, Nepal.
[2]Plant Quarantine and Pesticide Management Centre, Lalitpur, Nepal.
[3]Nepal Agricultural Research Council, Nepal.
[4]Beijing Ecoman Biotech Co. Ltd., China.
[5]Center for Molecular Dynamics Nepal and
[6]The University of Queensland, Australia.

***CORRESPONDING AUTHOR:**

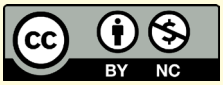
Debraj Adhikari
Email: adhikari.debraj1@gmail.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI : https://doi.org/10.3126/njst.v21i2.67158

Date of Submission: Oct 7, 2022

Date of Acceptance: Sep 25, 2023



ABSTRACT

An accurate identification at the species level is often the first step in successfully controlling, mitigating and managing of insect pests. Species identification utilizing molecular approaches can complement morphological identification, often resulting more accurate result. Tephritid fruit fly insects can be identified quickly using DNA barcoding technology. In this study, Chinese fruit fly *(Bactrocera minax)*, a destructive citrus pest collected in Nepal, was identified using barcoding method with the sequence of mitochondrial cytochrome c oxidase I (COI) gene.

Keywords: *Bactrocera minax*, Chinese citrus fly, DNA barcode

1. Introduction

Fruit flies of the Tephritidae family in Diptera order are the most damaging agricultural pests, especially in horticultural crops (tree fruits and fruit vegetables) (Vargas *et al.* 2015). Numerous fruit fly species have similar, overlapping, or identical features, making definitive morphological identification difficult or impossible (DeMeyer *et al.* 2015). Even though some fruit fly species appear to be morphologically identical, they may have distinct characteristics, host plants preferences, and genetic make-ups (Virgilio *et al.* 2019; Gomez-Cendra *et al.* 2016). Fruit fly damage is often associated with fruit drops, quality deterioration and inedible products. Apart from direct fruit loss, severe quarantine requirements to prevent exotic fruit fly species from entering also use a lot of resources of importing countries (Ekesi 2012). However, despite all the strict quarantine efforts, tephritids, particularly *Bactrocera* spp., continues to spread globally even to strategic pest-free areas (Koohkanzade *et al.* 2018).

Fruit flies of *Bactrocera, Zeugodacus,* and *Dacus* are mainly found inflicting significant damages in tree fruits and vegetable

fruits productions in Nepal. Among different fruit fly species reported from Nepal, the Chinese citrus fly has found the most serious damage in citrus orchards of Nepal (Adhikari *et al.* 2020; Adhikari & Joshi 2018). Rapid and reliable insect pest identification and diagnosis is often the most important information for the containment and mitigation of pest damages, and, in this respect, molecular techniques have shown some promising results. There are several molecular markers that can be utilized to identify tephritid species (Ochando *et al.* 2003; Douglas & Haymer 2001), and various techniques such as DNA barcoding and RFLP are often the preferred diagnostic tools in the present context (Chua *et al.* 2009).

Bactrocera minax was first collected in December 1984 from a sweet orange in Helambu, Sindhupalchok district, Nepal (Joshi & Manandhar 2001). The identified fruit flies specimens as *B. tsuneonis* are displayed in the Reference Museum of National Entomology Research Center (NERC), Nepal Agricultural Research Council at Khumaltar, Lalitpur. Later, on September 26, 2007, Dr. Gary J. Steck, Curator of Diptera, Florida State Collection of Arthropods, Florida, USA corrected the identity of *B. tsuneonis* of NERC to *Bactrocera minax* (Paudyal *et al.* 2016; Joshi 2019). The Chinese citrus fly (*B. minax*) is morphologically similar to the Japanese fruit fly (*B. tsuneonis*) (Drew & Romig 2013), but it lacks anterior supra-alar setae (EPPO 2021). Because of that, the prior specimen (EPPO/CABI 1996) was misidentified as *B. tsuneonis* rather than *B. minax*. In Nepal, characterizing studies of fruit fly species in molecular level are uttermost limited. In this study, the morphologically identified *B. minax* specimens are verified in the light of DNA barcoding analysis.

2. Materials And Methods

2.1 Collection of Specimens and Morphological Identification

Maggots infested sweet orange fruits (variety: Sindhuli Local) were collected in early November 2020 in Golanjor-5, Khaniyakharka (latitude: 27°17.145' N, longitude: 85°58.675' E, altitude: 1341 masl), Sindhuli (Fig. 1). Infested fruits were cut opened to expose maggots. Mature larvae (n = 200) were then collected and placed in 10 plastic containers (dimension: 15 cm height and 10 cm circumference) filled with garden soil (loamy soil of 20.7% average moisture content) 20 prepupae in each container. Pupation of these collected larvae took place at the experiment site. Jar's opening was closed with the help of a muslin piece and a rubber band to avoid larval escape. Soil in the jar was stirred after two months; pupae were examined, counted and recorded. Pupae placed in the same containers, fastened with a piece of nylon mesh. All the emerged adult fruit flies (n = 180) were morphologically identified as Chinese citrus fly in May (2021). Morphological characteristics of these fruit fly species has been described by Adhikari & Joshi (2018). Five dry fruit fly specimens (Fig. 2) out of the identified specimens were sent to the Center for Molecular Dynamics Nepal laboratory for DNA barcoding analysis. Since all the five fruit fly specimens were morphologically identical, only one representative sample was taken for molecular study.

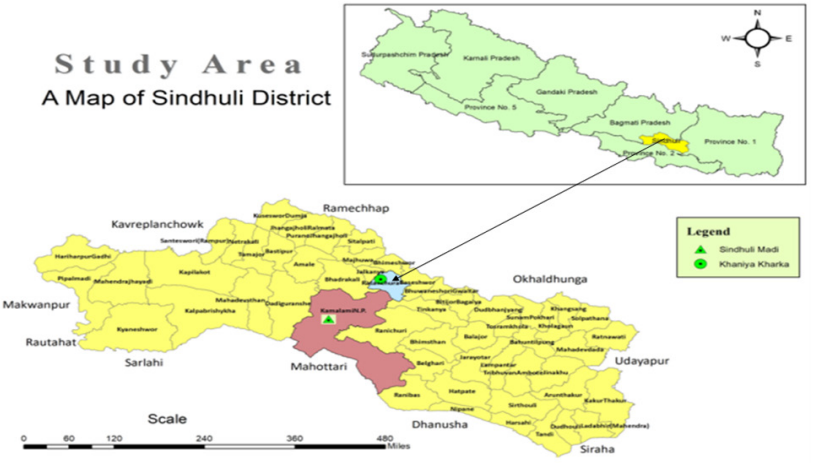


Fig. 1: Map of study site: Golanjor-5, Khaniyakharka, Sindhuli, Nepal.

Fig. 2: Fruit fly specimen, Bactrocera minax (a: male and b: female).

2.2 DNA Barcoding of Fruit Fly: For DNA barcoding of Chinese citrus fly the partial mitochondrial cytochrome c oxidase subunit I (COI) gene was used (Hebert *et al.* 2013).

2.3 DNA Extraction: DNA was extracted from the sample obtained by chopping specimens into small pieces and lysed with Lysis buffer and Proteinase K (56 °C for 10 hours). DNA extraction was conducted using GeneAll Exgene™ Tissue SV kit following the manufacturer's protocol (GeneAll® Exgene TM Protocol) with slight modification in lysis step using an additional CTAB lysis buffer to dissolve the exoskeleton. Final DNA was eluted in 50 µL TE buffer in order to obtain the concentrated DNA.

2.4 PCR of COI Mitochondrial Gene Marker: PCR was carried out in a 25 µL reaction volume-consisting 12.5 µL multiplex master mix (Qiagen, Germany), 5.0 µL Q solution (Qiagen, Germany), 4.50 µL of RNAse free water (Qiagen, Germany), 1.0µL of each forward and reverse primer and 1 µL of undiluted extracted DNA. Water based negative control to rule out any contamination was also used. Thermo cycling condition was slightly modified for this PCR (Table 1) (Hebert *et al.* 2013). Fig. 3 shows the Agarose Gel Electrophoresis of COI PCR Product from Chinese citrus fruit fly.

The Primer Pair used were:

Forward: LCO1490:
5'-GGTCAACAAATCATAAAGATATTGG-3'

Reverse HCO2198:
5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

Table 1. Thermo cycling condition for PCR

Steps	Temp. (°C)	Time (min)	Cycles
Initial denaturation	95	15	x1
Denaturation	94	1.0	x5
Annealing	45	1.5	
Extension	72	1.5	
Denaturation	94	1.0	x35
Annealing	50	1.5	
Extension	72	1.0	
Final extension	72	5.0	x1
Cool down	04	forever	x1

2.5 Agarose Gel Electrophoresis: PCR products were visualized in 1.5% agarose gel electrophoresis. A 100 bp DNA ladder from Solis Biodyne was used as reference. The expected PCR amplicon was nearly 650 bp. (Source: Hebert *et al.* 2013)

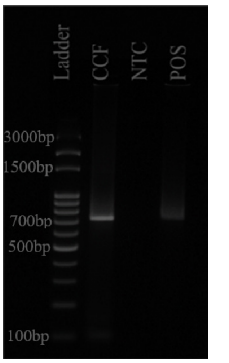


Fig. 3: Agarose Gel Electrophoresis of COI PCR Product from citrus fruit fly DNA.

Production of Barcode

The amplified PCR product (amplicon) was purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermofisher, Catalog No. 78200.200.UL) for 30 minutes at 55°C to digest the unused primers, followed by deactivation step at 85°C for 10 minutes. The purified PCR product was then sequenced on ABI thermocycler using BigDye™ Terminator V3.1 Cycle Sequencing Kit (Catalog No. 4337455) as per the manufacturer's protocol. Excess salts and dye terminators were removed using BigDye® XTerminator™ Purification Kit (Catalog No.4376486) following the manufacturer's instruction. The sample was then loaded on ABI 310 Genetic Analyzer for sequencing. The sequence was annotated using BLAST programme in NCBI database. Tt was then submitted at NCBI GenBank and a unique accession number was obtained for the sequence. Using Barcode of Life Data (BOLD) System v4 (boldsystems.org), a barcode from the DNA sequence has been produced (Fig. 5).

3. RESULTS AND DISCUSSION

3.1 Sequence Electropherogram

The portion of sequence electropherogram as produced by Sanger Sequencing for the COI marker in fruit fly sample is presented in Fig. 4.

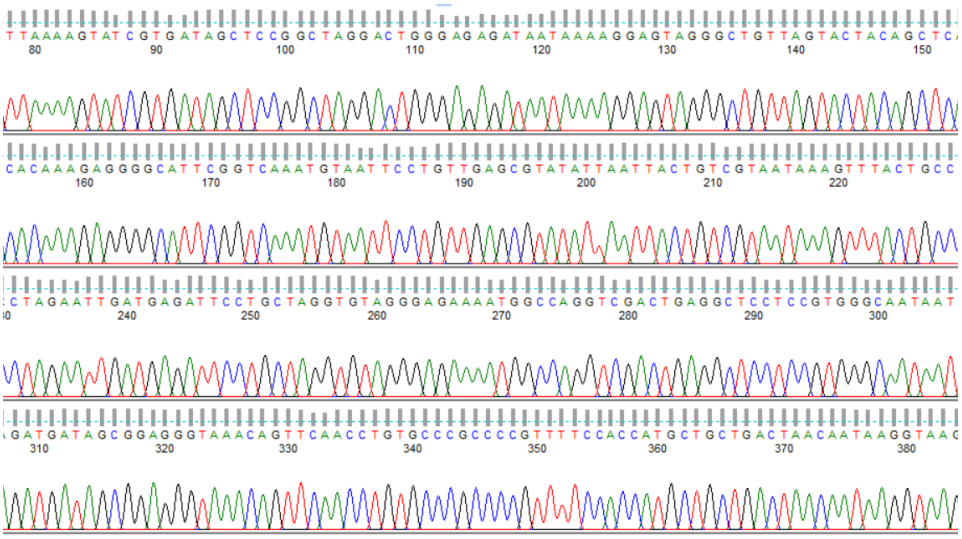


Fig. 4: Portion of sequence electropherogram as produced by Sanger Sequencing for the COI marker in fruit fly sample.

3.2 Sequence Fasta and Barcode

The electropherogram represented the sequence of COI marker sequenced in Sanger that was further converted to FASTA format for analysis. Fig. 5 shows the barcode.

>ON619567.1 *Bactrocera minax* isolate CCF1/INPL/NPL cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial

Fig. 5: DNA Barcode of Bactrocera minax generated by BOLD System v4.

The sequence can also be accessed in NCBI Genbank with accession no. ON619567.1. Basic Local Alignment Search Tool (BLAST) was used to perform the homologous nucleotide search for taxonomic identification. The highly similar sequences search for the obtained sequence showed the sequence to be most likely *Bactrocera minax* as shown in Table 2.

Table 2: NCBI BLAST result of the sequence with most significant alignment

Query length (bp)	BLAST matched Accession	Scientific Name	Common Name	QueryCoverage	E value	Percent identity
620	KU985287.1	*Bactrocera minax*	Chinese citrus fly	98%	0	99.67%

Sanger sequencing has been employed in this study to perform the molecular confirmation of the suspected citrus fruit fly with a remarkable 99.67% identity. Various other approaches have also been used for the molecular characterization of citrus fly. Lin *et al.* (2007) applied the PCR-RFLP analysis of mitochondrial and ribosomal DNA to develop a quick molecular diagnostic approach for the identification of *Bactrocera (Tetradacus) tsuneonis* and *Bactrocera (Tetradacus) minax*. These two species were separated based on their restrictive patterns using four primer pairs and five restriction endonucleases. They designed 4 primer pairs targeting the 4 different regions of cry1 genes and following the amplification of cry1 genes, they performed restriction digestion using 5 different restriction endonuclease to obtain variable number of bands. Observing the pattern of bands in electrophoresis they differentiated the *Bactrocera* species. In this study COI gene was specifically targeted due to its extensive research history across wide range of organisms making it suitable for taxonomic identification across broad spectrum of insect species (Zenker *et al.* 2020). The COI gene has a vast amount of reference sequence data available in databases like the Barcode of Life Data Systems (BOLD) and GenBank, which aids in the identification process (López *et al.* 2023). These databases provide a comprehensive collection of COI sequences for numerous insect species, making it easier to compare and match unknown sequences to known species. Other genes like cry1 gene may have limited reference sequence data available, particularly for non-target insect species. In addition, Sanger sequencing provides a high level of resolution and accuracy in determining DNA sequence of target gene, enabling single base pair differences, insertions and deletions, allowing for precise identification and differentiation of closely related species (Cheng *et al.* 2023). Hence, Sanger sequencing using COI gene provides a robust and standardized approach for DNA barcoding and species discrimination. This rapid and cost effective tool for identification of an organism can be very critical in the surveillance and management of fruit files.

4. Conclusion

Molecular identification of Chinese citrus fly is performed for the first time in Nepal. It is a rapid and a cost effective tool of identification of an organism. For effective surveillance and management techniques, especially in the case of fruit flies, species identification is crucial. By correct diagnosis of the Chinese citrus fly, specific control strategies can be developed to prevent its expansion and reduce damage to citrus crops. Additionally, this knowledge can help with quarantine operations and protect the agriculture sector.

Acknowledgements

The first author is thankful to the Agriculture and Forestry University, Chitwan, for prevailing the academic environment and University Grants Commission, Sanothimi, Bhaktapur, Nepal for support to conduct this research on Chinese citrus fly. Support from Prime Minister Agriculture Modernization Project, Project Implementation Unit, Sindhuli to collect specimens and Center for Molecular Dynamics Nepal laboratory for molecular study are highly acknowledged.

References

Adhikari, D., and S. L. Joshi, 2018. Occurrences and field identities of different species of fruit flies in sweet orange (*Citrus sinensis*) orchards in Sindhuli, Nepal. *Journal of Natural History Museum,* Nepal, 30, 47-54.
DOI: 10.3126/jnhm.v30i0.27511

Adhikari, D., R. B. Thapa, S. L. Joshi, X. H. Liang, and J. J. Du, 2020. Area-wide control program of Chinese citrus fly *Bactrocera minax* (Enderlein) in

Sindhuli, Nepal. *American Journal of Agricultural and Biological Sciences,* 15: 1-7.
DOI: 10.3844/ajabssp.2020.1.7

Cheng, C., Z. Fei, and P. Xiao, 2023. Methods to improve the accuracy of next-generation sequencing. *Frontiers in Bioengineering and Biotechnology*, *11*(January), 1–13.
DOI: 10.3389/fbioe.2023.982111
PMID:36741756 PMCID:PMC9895957

Chua, T. H., Y. V. Chong, and S. H. Lim, 2009. Species determination of Malaysian *Bactrocera* pests using PCR-RFLP analyses (Diptera: Tephritidae). *Pest Management Science., 66*(4), 379- 384.
DOI: 10.1002/ps.1886
PMID:19946858

DeMeyer, M., A. R. Clarke, M. T. Vera and J. Hendrichs, 2015. Editorial: resolution of cryptic species complexes of tephritid pests to enhance SIT application and facilitate international trade. *ZooKeys*, *540*,1–3.

Douglas, L. J., and D. S. Haymer, 2001. Ribosomal ITS1 polymorphism in *Ceratitis capitata* and *Ceratitisrosa* (Diptera: Tephritidae). *Annuals of Entomological Society of America, 94*,726–731.DOI: 603/0013-8746(2001)094[0726:RIPICC]2.0.CO;2

Drew, R. A. I., and M. C. Romig, 2013. *Tropical fruit flies (Tephritidae: Dacinae) of South-East Asia*. CAB International, Wallingford (UK).
DOI: 10.1079/9781780640358.0000

Ekesi, S., 2012. Combating fruit flies in Eastern and Southern Africa (COFESA): elements of a strategy and action plan for a regional cooperation program. https://www.yumpu.com/en/document/view/41335293/combating-fruit flies-in-eastern-and-southern-africa-the-global

EPPO, 2021. *Bactrocera minax.* EPPO datasheets on pests recommended for regulation.

EPPO/CABI, 1996. *Bactrocera tsuneonis*. In: *Quarantine Pests for Europe*. 2nd edition (Ed. by Smith, I.M.; McNamara, D.G.; Scott, P.R.; Holderness, M.). Cab International, Wallingford (UK).

GeneAll® Exgene TM Protocol Handbook 1 exgene Handbook for DNA PURIFICATION HANDBOOK GeneAll® Exgene TM Blood SV mini (105-101, 105-152) GeneAll® Exgene TM Clinic SV mini (108-101, 108-152) GeneAll® Exgene TM Cell SV mini (106-101, 106-152) AmpONE TM, Exfection TM, Exgene TM, Expin TM, Exprep TM, EzClear TM, EzSep TM, EzPure TM, GenEx TM, Hybrid-Q TM, DirEx TM, Allspin TM, RiboEx TM, Riboclear TM, Ribospin TM are trademarks of. (n.d.).

Gomez-Cendra, P. V., L. E. Paulin, L. Orono, S. M. Ovruski and J. C. Vilardi, 2016. Morphometric differentiation among *Anastrepha fraterculus* (Diptera: Tephritidae) exploiting sympatric alternate hosts. *Environ. Entomol., 45*, 508–517.
DOI: 10.1093/ee/nvv224
PMID:26787122

Hebert, P. D. N., J. R. deWaard, E. V. Zakharov, S. W. J. Prosser, J. E. Sones, J. T. A. McKeown, and J. La Salle, 2013. A DNA 'Barcode Blitz': Rapid Digitization and Sequencing of a Natural History Collection. *PLOS ONE,* 8(7), e68535. https://doi.org/10.1371/JOURNAL.PONE.0068535
PMID:23874660 PMCID:PMC3707885

Joshi, S. L., and D. N. Manandhar, 2001. Reference insects of Nepal. Entomology Division, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal. 1-122. pp.

Joshi, S. L., 2019. *Bactrocera minax* (Enderlein) (Diptera: Tephritidae) and its invasion in Nepal. Paper presented on National Workshop on Chinese citrus fly (*Bactrocera minax*) on June 13, 2019 at Khumaltar, Lalitpur. Prime Minister Agriculture Modernization Project, Project Management Unit, Khumaltar, Lalitpur.

Koohkanzade, M., M. Zakiaghl, M. K. Dhami, L. Fekrat and H. S. Namaghi, 2018. Rapid identification of *Bactrocera zonata* (Dip.: Tephritidae) using TaqMan real-time PCR assay. *PLoS ONE, 13*(10), e0205136.
DOI: 10.1371/journal.pone.0205136
PMID:30286152 PMCID:PMC6171934

Lin, L., J. Wu, L. Zeng, G. Liang, X. Hu, and R. Mo, 2007. Rapid identification of two species of Tetradacus by PCR-RFLP. *Chinese Bulletin of Entomology*, 44(4): 588-592.

López, L. P., B. L. Rodrigues, I. D. Velez, and S. Uribe, 2023. Improving the COI DNA barcoding library for Neotropical phlebotomine sand flies (Diptera : Psychodidae). *Parasites & Vectors*, 1–12.

DOI: 10.1186/s13071-023-05807-z
PMID:37308979 PMCID:PMC10259023

Ochando, M. D., A. Reyes, C. Callejas, D. Segura, and P. Fernandez, 2003. Moleculargenetic methodologies applied to the study of fly pests. *Trends in Entomology., 3*,73–85.

Paudyal, K. P., T. N. Shrestha, and C. Regmi, 2016. Citrus Research and Development in Nepal, Six Decades of Horticulture Development in Nepal. *Silver Jubilee Special. Nepal Horticulture Society*, Lalitpur, Nepal.113-144.

Vargas, R. I., J. C. Piñero, and L. Leblanc, 2015. An Overview of Pest Species of *Bactrocera* Fruit Flies (Diptera: Tephritidae) and the Integration of Biopesticides with Other Biological Approaches for Their Management with a Focus on the Pacific Region. *Insects*, *6*(2), 297–318.
DOI: 10.3390/insects6020297
PMID:26463186 PMCID:PMC4553480

Virgilio, M., J. H. Daneel, A. Manrakhan, H. Delatte, K. Meganck, and M. DeMeyer, 2019. An integrated diagnostic setup for the morphological and molecular identification of the *Ceratitis* FAR complex (*C. anonae*, *C. fasciventris*, *C. rosa*, *C. quilicii*, Diptera, Tephritidae). *Bull. Entomol. Res., 109*, 376–382.
DOI: 10.1017/S0007485318000615
PMID:30203730

Zenker, M. M., A. Specht, and V. G. Fonseca, 2020. Assessing insect biodiversity with automatic light traps in Brazil: Pearls and pitfalls of metabarcoding samples in preservative ethanol. *Ecology and Evolution*, *10*(5), 2352–2366.
DOI: 10.1002/ece3.6042
PMID:32184986 PMCID:PMC7069332

Enhancement of Secondary Radiation Flux Energy 3.63 % During Appearance of Full Moon on October 13, 2022 at Udaipur (Rajasthan), India

Devendra Pareek[1*] and Deepali Baregama[1]

[1]Department of Physics, Bhupal Nobles' University, Udaipur, 313001, India

***CORRESPONDING AUTHOR:**

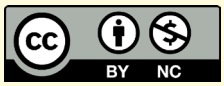
Devendra Pareek
Email: deven.pareek69@gmail.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI : https://doi.org/10.3126/njst.v21i2.67159

Date of Submission: Apr 3, 2023

Date of Acceptance: Sep 25, 2023



ABSTRACT

We observed variation of secondary radiation flux energy (SRFE) at Udaipur (27° 43' 12.00" N, 75° 28' 48.01" E), Rajasthan, India during Full Moon in month of October, 2022. In this experimental study ground based NaI (Tl) Scintillation detector used. The data files were stored in computer for half hour duration around time 21.00 IST to 21.30 IST on October 6, 9, 10, 11, 12, 13, 14 and 15. The objective in this study is to observe secondary radiation flux energy during Full Moon. Analyzed data reveal significant enhancement of secondary radiation flux energy about 3.63 % on date of full Moon. We interpret such variation of SRFE on the basis of more reflection of solar radiation form surface of Full Moon.

Keywords: Cosmic radiation, Solar radiation, Reflected solar radiation, Secondary radiation flux energy.

1. Introduction

Experimentally explicated cosmic rays are inherent high-energy charged particles, experimental outcomes revels that these are nuclei of atoms ranging from the lightest to the heaviest elements in the periodic table and move through space at nearly the speed of light (Longair 1992; Chaisson *et al.* 1990; Mewaldt 2010) Primary cosmic radiation lies in the energy range from 10^9-10^{20}eV or more (Kudela 2009). Simpson 1983 gave information about cosmic radiation's chemical abundances in different energy ranges, compared solar system abundances and estimated abundances for the local interstellar medium. About 89% of these are protons, 10% of nuclei of helium, and about 1% of others heavier elements. Also high energy electrons, positrons, and other subatomic particles are present originate in outside the solar system, distributed throughout the Milky Way galaxy. The intensity of primary cosmic radiation flux above50 km from the surface of the Earth remains almost the same. Primary cosmic radiation produces denser ionization about 20 km from the Earth's surface, which is called "secondary" particles and called secondary cosmic radiation (Carl *et al.* 1936). These particles have X- rays, protons, alpha particles, pions, muons, electrons, neutrinos and neutrons. These particles increase rapidly as

these moves downward in the atmosphere. After each interaction, the particles lose energy (Bhabha 1938a; Bhabha 1938b). In this way, secondary cosmic particles shower down through the atmosphere to the Earth's surface (Allkofer *et al.* 1984). Secondary radiation has three components (A) electromagnetic components (B) hadronic component and (C) masonic component (Walter Heinrich 1937; Nordheim 1937). The electromagnetic component has electrons and gamma particles. Hadronic component has low energy protons and neutrons. In contrast, masonic components have pions, muons. Therefore, penetrating primary cosmic radiation produced a secondary shower (Heitler 1938). This secondary radiation flux can be detected using appropriate detector on the ground (Kodama 1983; Chilingarian et *al.* 2010). A galaxy, a star, or a cluster of galaxies produces gravitational lensing effect (Mellier 1998; Narayan & Bartelmann 1996). A. S. Eddington and collaborators proved this phenomenon in a famous experiment during total solar eclipse in 1919. To observe secondary radiation flux, many scientist groups conducted experimental studies during normal days and on days of special celestial events such as Solar eclipse, Lunar eclipse, the appearance of a comet in the sky, phases of the Moon, the closest approach of celestial objects, transit of celestial objects etc. with the help of the efficient counter system.

Solar eclipse studies carried out to observe radiation flux (Bhattacharya *et al.* 1997; Kandemir *et al.* 2000; Bhaskara *et al.* 2011; Pareek *et al.* 2013). Lunar eclipses experimental studies were conducted by (Pareek *et al.* 2013; Raghav *et al.*2011; Rao *et al.* 1967). Using scintillation counter experimental study of the comet was conducted by (Pareek *et al.* 2014) in the energy range of 10 keV to 5 MeV. Analyzed data showed an unusual variation of secondary cosmic radiation flux at the energy about 1.127 MeV, 2.29 MeV and 3.66 MeV. Pareek *et al.* 2012 conducted an experimental study for celestial event transit of Venus 6thJune, 2012 at Udaipur, India. After analyzing it was observed 2% decrement in secondary solar radiation gamma ray flux. In September 2000 the lunar phases ground-based experimental study was conducted by Pareek *et al.* 2014 using a Scintillation counter. During the passes of the Moon through the background of the Capricornus constellation, an abrupt change in the energy spectra was noticed on 9th and10th September 2000 due to the gravitational lensing effect. Pareek *et al.* 2022 conducted an experimental study for the transit of the Sun across Constellations Libra, Virgo. The analyzed result showed a variation of Secondary Gamma Radiation Flux in Months November 2018 and September 2019 respectively at Udaipur, India. Also, for transit of the sun across constellation Libra in October and November 2020 at Udaipur Pareek *et al.* 2021 conducted an experimental study and observed the same result of variation of secondary gamma radiation flux.

Pareek *et al.* 2022 conducted experimental studies for transit of Moon in different constellations. Analyzed data showed variation of secondary radiation flux during these events. Another experimental study of change of Moon position in sky was carried and showed the variation of secondary gamma radiation flux in the month November, 2020 at Udaipur (India) (Pareek 2022).

To unveil the hidden secrets of high energy astronomy, technical advances, over more than half century, have been achieved so that we could able to pinpoint how astronomical observations and physical concepts interact. For this purpose, a large number of experimental studies carried out to collect good quality data of CR (Cosmic radiation) and SEP (Solar energetic particles) with the help of advanced technologies by astronomers, for different celestial events occurring at various points of time. However due to these events, it is found that the characteristics of GCR and SEP are modulated and manifested in the ground based observation for the terrestrial secondary radiation (SR) flux. These SR signals carrying the signatures of modulated GCR and SEP are measured by efficient detectors. In this experimental study, we want to see the effect on secondary flux due to appearance of Full Moon at Udaipur India.

2. Methodology

Experimental Set-up and Observations:

Scintillation detector Model SD 152 F flat type (Fig. 1) (make: Nucleonix) was employed to detect secondary radiation flux (SR) produced by the SEP and CR. The secondary radiation flux was incident on a NaI (Tl) of 2" x 2" diameter optically coupled with photo multiplier tube (PMT). This PMT is connected with USB interface with multi-channel analyzer (MC 1000) having 1024 channels built-in high voltage and shaping amplifier. The entire integrated assembly was used to collect secondary radiation flux and the detector pointed in the line of site of the Moon. This counter system was used to collect the counts as a function of

time. The scintillation detector was kept on the terrace of Astronomy Laboratory of Department of Physics, Bhupal Nobles' University Udaipur (Rajasthan) India. The data files were stored in computer for half hour duration around time 21.00 IST to 21.30 IST on October 6, 9, 10, 11, 12, 13, 14 and 15.

3. Results and Discussions

The dates of observation were October 6, 9, 10, 11, 12, 13, 14 and 15. As depicted in Fig.s. 2 and 3 the panels of SR flux data files between energy and counts were taken around time from 21.00 IST to 21.30 IST.

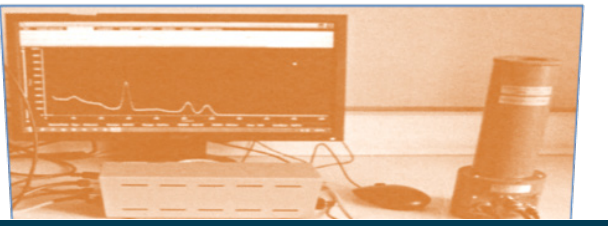


Fig. 1: Scintillation Counter Systam.

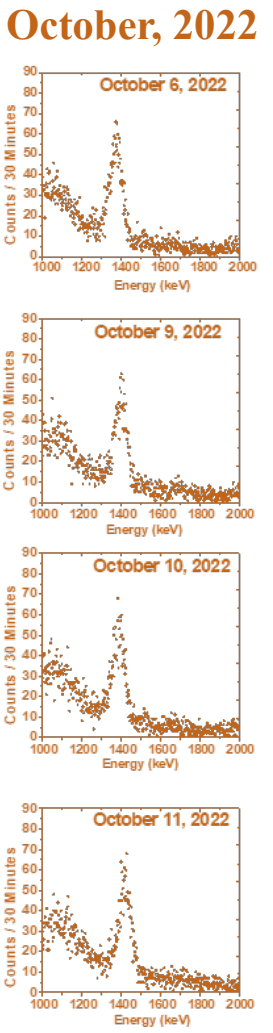


Fig. 2: Panels of secondary radiation flux energy and counts

October, 2022

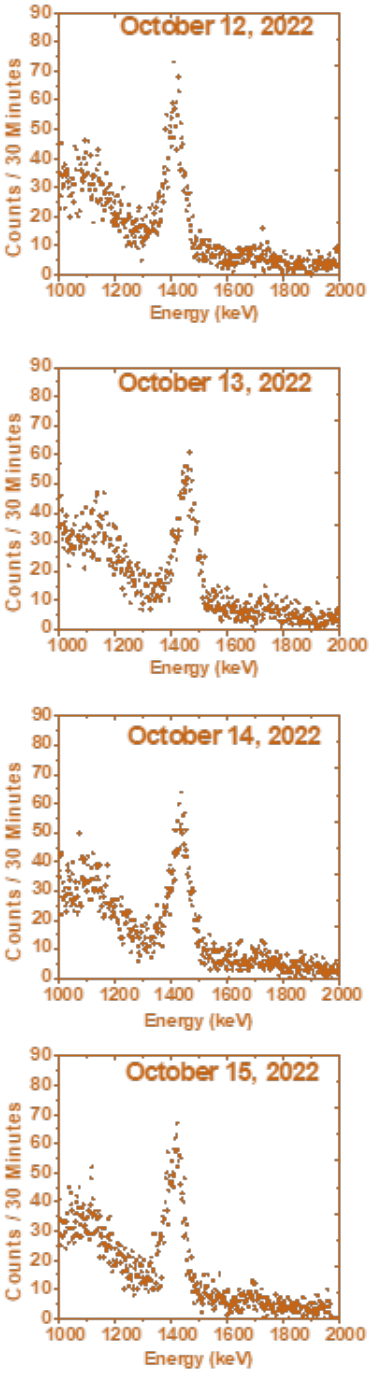


Fig. 3: Panels of secondary radiation flux energy and counts

Fig 2 and 3 show the existence of specific peaks. We used Lorentz peak fit concept in order to understand the characteristics and energy variation of SR flux peaks in the energy range from 1300 keV to 1600 keV as shown in below Fig.s. 4 and 5.

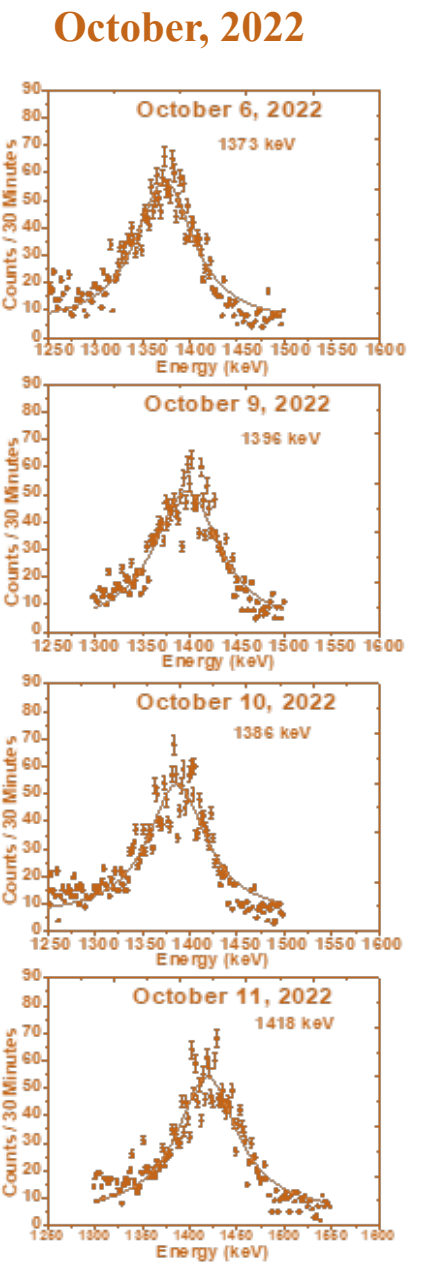


Fig. 4: Panel of energy peak of secondary radiation flux and counts

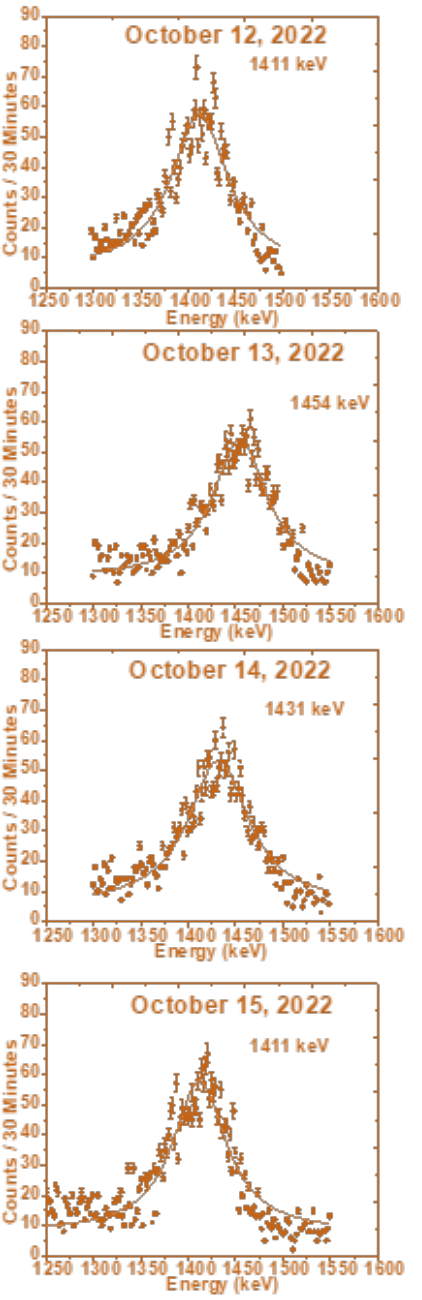


Fig. 5: Panel of energy peak of secondary radiation flux and counts

Using above panels of SR flux energy and counts files, we made Table 1 which represents secondary radiation flux energy with respect to dates in month of October, 2022 for half an hour.

Table 1 (Peak energy of secondary radiation flux)

Sr. No.	Date	Energy (keV)
October		
1	6	1373
2	9	1396
3	10	1386
4	11	1418
5	12	1411
6	13 (Full Moon)	1454
7	14	1431
8	15	1411

Using Table 1 we made following Fig. 6 between date and energy in keV for month of October, 2022

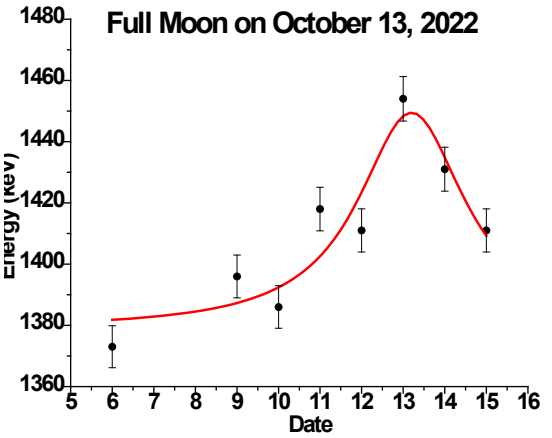


Fig. 6: Secondary radiation flux energy with date

Above Fig. 6 and Table 1 represent that in Month of October (2022) secondary radiation flux energy were less on comparison to October 13, 2022 (Full Moon). On October 13, 2022 highest energy 1454 keV we observed. In this study from Fig. 6 and Table 1, we conclude that on date October 13, 2022 there was significant enhancement of secondary radiation flux energy i.e. during appearance of Full Moon. Observed energy during Normal dates were: on October 6 - 1373 keV, 9–1396 keV, 10 - 1386 keV, 11 – 1418 keV, 12 – 1411 keV, 14 – 1431 keV and 15- 1411 keV. When we average all normal dates secondary radiation flux energy then it is equal to 1403 keV.

To see the enhancement in secondary radiation flux energy on the October 13, 2022 we used the following formula:

$$\% \text{ of Enhancement of Energy} = \frac{\text{Energy on October 13, 2022} - \text{Average of energy on normal dates}}{\text{Average of energy on normal dates}} \times 100$$

Using above formula, we observed about 3.63% Enhancement in secondary radiation flux energy on October 13, 2022.

4. Conclusion

I conducted three experimental studies related to Moon to observe secondary radiation flux and energy. The first experimental study was Phases of Moon in month of September, 2000 (Pareek *et al.* 2014). In this experimental I observed variation of secondary radiation flux energy during different Phase of Moon. Another study was carried out transit of Moon in different constellations, results of this experimental study showed variation of secondary radiation flux during transit of Moon in different constellations (Pareek *et al.* 2022). Third study was conducted change of position of Moon in sky. Results of this experimental study showed variation of secondary radiation flux due to change of position of Moon in sky. From November 1 to 7 the integrated counts of secondary gamma radiation were regularly increasing. On date November 7 we observed highest counts. The probable reason is, as changes of position of Moon in sky and approaching towards the detector more reflected solar radiation reached. Therefore formation of secondary shower was more (Pareek 2022).

Getting inspired on previous experimental studies related to Moon, this experimental study was conducted for astronomical event Full Moon to observe secondary radiation flux energy.

The probable reason for this unique and first-time finding is as follows:

(A) During of the appearance of Full Moon more intense reflected solar radiation entered in the atmosphere of Earth. Due to this more secondary radiation flux energy formed in the atmosphere as we observed on surface of Earth during Full moon.

Significant enhancement of secondary radiation flux energy about 3.63% we observed on date October 13, 2022. Such unique enhancement we can understand with help of above reason. The Moon is reflector of Solar Radiation and at time appearance of Full Moon more intense radiation reflected from Moon surface. This experimental study gave conclusion that during Full Moon event on surface of Earth significant enhancement of secondary radiation flux energy we observed.

Acknowledgements

The authors are thankful to Aaryan Mitra and Prateek Ninama who were participated in this experimental study.

References

Allkofer, O. C., and P. K. F. Grieder, 1984. Cosmic Rays on Earth. https://inis.iaea.org/search/search.aspx?orig_q=RN:15054748

Bhabha, 1938a. Nuclear forces, heavy electrons and the β-decay. Proc. Roy.Soc. Lond. A 166(1938) 501. DOI: 10.1098/rspa.1938.0107

Bhabha, 1938b. Nuclear forces, On the theory of heavy electrons and nuclear forces, Nature 141 117 DOI: 10.1038/141117a0

Bhaskara, A., A. Purohit, M. Hemalatha, C. Pai, A. Raghav, C. Gurada, S. Radha, V. Yadav, V. Desai, A. Chitnis, P. Sarpotdar, and A. Patankar, 2011. A study of secondary cosmicray flux variation during the annular eclipse of January 15 2010 at Rameswaram, *India, Astroparticle Physics, Volume 35,* Issue 5, Pages 223-229. DOI: 10.1016/j.astropartphys.2011.08.003

Bhattacharyya, A., S. Biswas, B. K. Chatterjee, M. Das, P.K. Das, T. K. Das, T. K. De, M. H. Engineer, R. Mukherjee, Sibaji Raha, S. C. Roy, S. K. Saha, A. K. Sen, B. Sinha, and D. Syam, 1997. Variation of γ-Ray and Particle Fluxes At the Sea Level During the Total Solar Eclipse of October 24, 1995, *Astrophysics and Space Science volume* 250, pages313–326. DOI: 10.1023/A:1000408422978

Carl, D. A., and H. N. Seth, 1936. Cloud Chamber observations of Cosmic Rays at 4300 Meters Elevation and Near Sea-Level, Physical Review 50, 263. DOI: 10.1103/PhysRev.50.263

Chaisson, E., and S. Mcmillan, 1999. Astronomy today, 3rd Edition, Prentice Hall.

Chilingarian, A., A. Daryan, K. Arakelyan, A. Hovhannisyan, B. Mailyan, L. Melkumyan, G. Hovsepyan, S. Chilingaryan, A. Reymers, and L. Vanyan, 2010. Ground-based observations of thunderstorm-correlated fluxes of high energy electrons, gamma rays, and neutrons, Physical Review D, 82(4), 043009. DOI: 10.1103/PhysRevD.82.043009

Heitler, W., 1938. Showers produced by the penetrating cosmic radiation, Royal Society, Vol. 166, Issue 927. 1938. Page(s): 529-543. DOI: 10.1098/rspa.1938.0108

Kandemir, G., 2000. The Last Total Solar Eclipse of the Millennium in Turkey, ASP Conference Series, Vol. 205.

Kodama, M., 1983. Ground Albedo Neutrons Produced by Cosmic Radiations, *Physical Society of Japan, Journal,* 52, 1503-1504. DOI: 10.1143/JPSJ.52.1503

Kudela, K., 2009. On energetic particles in space. Actaphysicaslovaca 59,537-652. DOI: 10.2478/v10155-010-0098-4

Longair, M. S., 1992. High energy Astrophysics 1second edition Pub. Cambridge Universitypress.

Mellier, Y., 1998. Porbing the universe with weak lensing. https://arxiv.org/abs/astroph/9812172.

Mewaldt, R. A., cosmic rays, 2010. CaliforniaInstitute of Technology http://www.rl.caltech.edu/personnel/dick/cos_encyc.html.

Nordheim, L. W., 1937. On the Absorption ofCosmic-Ray Electrons in the Atmosphere, Physical Review, 51, 1110. DOI: 10.1103/PhysRev.51.1110

Narayan, R., and M. Bartelmann, 1996. Lectures on gravitational lensing. https://arxiv.org/abs/astro-ph/9606001

Pareek, D., S. N. A. Jaaffrey, K. P. Talesra, R. Yadav, and S. Ameta, 2013. Experimental study of Variation of Secondary Cosmic Gamma Ray Flux and Energy during Partial Solar Eclipse of January 4 2011 at Udaipur, India, *Research journal of physical sciences,* Vol. 1(5), pages 22-30.

Pareek, D., and S. N. A. Jaaffrey, 2013. Experimental Study of Variation of Secondary Cosmic Gamma Ray Flux during Total Lunar Eclipse 4th April, 1996 and 16th July, 2000, *Research journal of physical sciences, Volume 1(4),* Page no. 22-27.

Pareek, D., and S. N. A. Jaaffrey, 1996. Experimental Study of Emission of X – Ray and Variation of Secondary Cosmic Ray Flux at Some Energy During the appearance of Comet Hyakutake March, *International Journal of scientific research, Ahmedabad, Volume 3,*Issue 4, April-2014 Page no. 411-413
DOI: 10.15373/22778179/APR2014/146

Pareek, D., S. N. A. Jaaffrey, H. T. Daspattnayak, and M. Shrimali, 2017. Variation of Secondary Cosmic Gamma Ray Flux duringVenus Transit on 6th June, 2012 at Udaipur, India, Global *Journal for research analysis, Ahmedabad, Volume 6*, Issue 5, Page no. 500-501

Pareek, D., and S. N. A. Jaaffrey, 2014. Experimental Study of Phases of Moon For Observing Variation of Secondary Cosmic Gamma Ray Flux, Energy and X-Ray Flux in the month of September 2000 at Udaipur, India, International *Journal of scientific research, Ahmedabad, Volume 3,* Issue 5, Page no. 6-10.
DOI: 10.15373/22778179/MAY2014/2

Pareek, D., and P. Sengar, 2022. Transit of the Sun across Constellations Libra, Virgo and Variation of Secondary Gamma Radiation Flux in November, 2018 and September, 2019 respectively at Udaipur, India. *Jordan journal of Physics* Vol 15(2) pages 195-199.
DOI: 10.47011/15.2.9
PMID:37284623

Pareek D., and P. Purohit, 2021. Transit of the Sun across constellation Libra and variation of secondary gamma radiation flux in October and November, 2020 at Udaipur, India. *Indian Journal of Science and Technology 14(8)*: 718-724.
DOI: 10.17485/IJST/v14i8.263

Pareek, D., and P. Sengar, 2022. Variation of secondary gamma radiation flux during closest approach of Mars towards Earth, Mars at opposition and transit of Moon across different constellations, planets in month of October, 2020 at Udaipur, India. *Jordan journal of Physics* Vol. 159 (4), pages 409-416
DOI: 10.47011/15.4.10
PMID:27486505

Pareek, D., 2022. Change of Position of Moon in Sky and Effect on Secondary Gamma Radiation Flux in Month of November, 2020 at Udaipur, India published in the International book "Sustainable Development for Society, Industrial Development, Material, Energy and Environment: Key Issues, Opportunities and Challenges"; I : 165-168, ISBN: 978-81-953847-8-5.

Raghav, A., A. Bhaskar, V. Yadav, N. K. Bijewar, C. Pai, A. Koli, N. Navale, G. Singh, N. Dubey, S. Pawar, P. Parab, G. Narvankar, V. Rawoot, V. Rawat, S. B. Nagnath, G. C. Rozario, N. Kaushal, S. K. Tiwari, and M.R. Press, 2011. Confirmation of secondary cosmic ray flux enhancement during the total lunar eclipse of December 10, *Journal of geophysical research: Space Physics/ Volume 118,* Issue, 10, 6426-6433.
DOI: 10.1002/2013JA019007

Rao, A. J. N., 1967. Variation in the background counting rate at Tirupati during the periods of solar and lunar eclipses, Physics Letters A, 25, 2, 74.
DOI:10.1016/0375-9601(67)90348-9

Simpson, J., 1983. Elemental and isotopic composition of alactic cosmic rays, annual reviews of nuclear and particle science, 33,323-381.
DOI: 10.1146/annurev.ns.33.120183.001543

Walter Heinrich H., 1937. On the analysis of cosmic rays, Proceeding of the Royal Society A.161, 261 .
DOI: 10.1098/rspa.1937.0145

Multi-drug Resistant *Staphylococcus aureus* and *Escherichia coli* in Marketed Raw Meat in Kathmandu Valley

Deepa Neupane[1], Joshna Thapa[1*], Sushil Neupane[1], Namrata Dulal[1], Sushma Kathet[1], Sudeep K.C[2], Santosh Khanal[1], and Janak Raj Dhungana[1]

[1]Department of Microbiology, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu 44600, Nepal.
[2]Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu.

***CORRESPONDING AUTHOR:**

Joshna Thapa
Email: zthapa99@gmail.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI :
https://doi.org/10.3126/njst.v21i2.67160

Date of Submission: Mar 15, 2023

Date of Acceptance: Jun 22, 2023

ABSTRACT

Antimicrobial resistance is a critical worldwide issue that affects both humans and animals. Antimicrobial resistance in microorganisms has been associated to the indiscriminate use of antibiotics in livestock and poultry. Bacterial antimicrobial resistance is witnessing a rapid rise in both human and animal health sector. Still antimicrobial resistance patterns in Nepal are poorly documented, with only a few studies on bacterial prevalence and antimicrobial resistance pattern have been done. This study characterized and provided antimicrobial resistance status of bacteria from raw meat sold in Kathmandu valley. This study was conducted with an objective to find the prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus* and *Escherichia coli* from raw meat sold in Kathmandu valley. The cross-sectional study was conducted from February to April 2022 at Nepalese Farming Institute, Dillibazar, Kathmandu. A total of 100 raw meat samples, chicken (n= 60), pork (n=20) and buff (n=20) were collected from different retail shops. The meat samples were minced and enriched in M-Staph broth and MacConkey broth at 37°C for 24 h. The suspension was sub-cultured on Mannitol salt agar and MacConkey agar. The bacterial isolates were identified on the basis of colony morphology, Gram's staining reaction and biochemical tests. Antibiotic susceptibility pattern of bacterial isolates were determined by modified Kirby-Bauer disc diffusion method according to Clinical & Laboratory Standards Institute (CLSI) guidelines. Out of 100 meat samples, 162 bacterial isolates were detected, where *E. coli* 51.85% (n=84) was the most prevalent bacteria followed by *S. aureus* 48.15% (n=78). Majority of *E. coli* isolates (63.09%) were resistant to tetracycline and most of *S. aureus* isolates (37.1%) were resistant to gentamicin. Multi-drug resistance was higher in *E. coli* (51.1%) than *S. aureus* (23.07%). Therefore controlled use of antibiotics in all sectors should be implemented and proper policy and regulations should be formulated on meat handling.

Keywords: *Escherichia coli*, Meat, Multi-Drug Resistance (MDR), *Staphylococcus aureus*

1. Introduction

Meat is one of the most important, nutrient-dense, and energy-dense natural foods consumed by humans to meet their daily nutritional needs. It is very important in keeping a healthy and balanced diet, which is necessary for achieving optimal human growth and development (Ahmad *et al*. 2018). Meat is susceptible to contamination at any phases, from primary manufacture to consumption (farm-to-fork). Contaminated meat is one of the most common causes of food-borne illnesses and death, as pathogens enter the body through ingestion (Bersisa *et al*. 2019).

Food-borne diseases have consistently been the world's leading source of sickness and death. Food-borne infections are becoming more well-known as they have an impact on both health and the economy. Poultry and other meats are one of the most important carriers for bacteria that cause disease (Bantawa *et al*. 2019). Nepal lacks proper policy and regulations for the use of antibiotics in poultry and agriculture which leads us to tackle with these bacteria immune of antibiotics. Multidrug resistant bacteria are far greater threat to humanity than they are considered.

Antibiotic resistance has been linked to the widespread use of non-therapeutic antibiotics in animals, according to growing evidence (Shrestha *et al*. 2017). Multidrug resistance (MDR) refers to bacteria's ability to withstand multiple kinds of antibiotics (three or more classes) that are structurally diverse and have various molecular targets. Misuse of antibiotics can result in bacterial resistance to antibiotics which raise the burden of chronic disease and increases health-care costs. Humans are exposed to resistant bacteria through direct contact with animals, exposure to animal waste, eating of raw meat, and contact with meat surfaces (Marshall & Levy 2011).

In the 21st century, antimicrobial resistance is the global health concern and is a top health challenge, with bacterial infections increasingly failing to antibiotics therapies. Burden of antimicrobial resistant bacteria affects the economy and health of people in both developed and developing countries. Therefore, in this study, we aim to investigate the presence of foodborne illness causing microorganisms in raw meat, along with their antibiotic resistance pattern. We expect the dissemination of our results would lead to improved hygiene among the meat vendors and updated policy and guidelines for the use of antimicrobial drugs.

2. Materials and Methods

2.1 Study site and study period

The research work was conducted in the microbiology laboratory, Nepalese Farming Institute, Kathmandu from February to April 2022.

2.2 Sample collection

In the study, 60 chicken meat (20 each from Kathmandu, Bhaktapur and Lalitpur), 20 pork and 20 buff meat (each 10 samples from Kathmandu, 5 from Bhaktapur and 5 from Lalitpur) were collected from 100 different retail vendors. The samples were collected in sterile vial and were transported to the laboratory while maintaining cold chain (4 °C).

2.3 Culture of specimens

The meat samples were minced and enriched in MacConkey broth and M-Staph broth and were incubated at 37 °C for 24 hours. After incubation, the suspensions were sub-cultured in Mannitol salt agar and MacConkey agar and were incubated 37 °C for 24 hours. The bacterial colonies were sub-cultured in Nutrient Agar (NA) on the following day.

2.4 Identification of bacterial isolates

The colony morphology on Mannitol salt agar and MacConkey agar were noted. The isolated colonies were subjected to Gram staining and different biochemical tests were performed for bacterial identification.

2.5 Antibiotic susceptibility test

Antibiotic susceptibility test of *S. aureus* and *E. coli* isolates was performed by modified Kirby-Bauer disc diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI 2021). Bacterial suspensions were prepared as per guidelines from the overnight grown culture in NA. The turbidity of the suspension was matched with McFarland Standard 0.5 and lawn culture of test bacterium was prepared on Mueller Hinton Agar (MHA). The antibiotic discs were placed on MHA plates with sterile forceps and the plates were incubated at 37 °C for 24 hours. After incubation, diameter of zone of inhibition was measured, and the results were interpreted as sensitive, intermediate, or resistant. Antibiotics used in susceptibility test were Ciprofloxacin (5 mcg), Co-trimoxazole (25 mcg), Ceftazidime (30 mcg), Cefotaxime (30mcg), Piperacillin-Tazobactam (100/10 mcg), Cefepime (30 mcg), Chloramphenicol (30 mcg),

Tetracycline (30 mcg), Imipenem (10 mcg), Ampicillin (10 mcg), Amikacin (30 mcg), Gentamicin (10 mcg), Aztreonam (30 mcg) for *E. coli* and Linezolid (30 mcg), Cotrimoxazole (25 mcg), Tetracycline (30 mcg), Cefoxitin (30 mcg), Gentamicin (10 mcg), Clindamycin (2 mcg), Ciprofloxacin (5 mcg), Erythromycin (15 mcg), Penicillin (10 mcg). *S. aureus* isolates, which were resistant to Cefoxitin (30 mcg), were considered as methicillin resistant. Clindamycin (2 mcg) and Erythromycin (15 mcg) antibiotics were used to detect inducible Clindamycin resistance in *S. aureus* by D-zone test confirmation.

2.6 Data Analysis

The data were entered in Microsoft Excel and frequency, distribution were analyzed accordingly.

3. Results

All buff meat samples were contaminated with *E. coli* followed by 49 (81.66%) from chicken whereas 42 (70%) *S. aureus* were recovered from chicken meat followed by buff and pork, each with 90% (Table 1).

Table 1: Distribution of bacterial isolates from different sampling sites

Samples	Sites	Culture positivity	
		E. coli N (%)	S. aureus N (%)
Chicken	Kathmandu	18 (90)	15 (75)
	Bhaktapur	14 (70)	15 (75)
	Lalitpur	17 (85)	12 (60)
Total		49 (81.66)	42 (70)
Buff	Kathmandu	5 (100)	5 (100)
	Bhaktapur	10 (100)	9 (90)
	Lalitpur	5 (100)	4 (80)
Total		20 (100)	18 (90)
Pork	Kathmandu	9 (90)	9 (90)
	Bhaktapur	3 (60)	4 (80)
	Lalitpur	3 (60)	18 (90)
Total		15 (75)	18 (90)
Grand Total		84 (84)	78 (78)

A total of 84 *E. coli* were isolated from various meat samples and all of them were sensitive to Imipenem, Aztreonam, Cefepime and Piperacillin-Tazobacatam. In case of chicken meat, 93.87% were resistant to Tetracycline followed by Ampicillin (77.55%) and Co-trimoxazole (75.51%). Similarly, in buff meat samples, 30% of bacterial isolates were resistant to Cefotaxime followed by Amikacin (15%). And, 40% of bacterial isolates from pork meat were resistant to Tetracycline and Co-trimoxazole (Table 2)

Out of total bacterial isolates, 78 of them were *S. aureus* and all of them were sensitive to Linezolid. 52.38% *S. aureus* isolated from chicken meat were resistant to Ciprofloxacin followed by Tetracycline with 47.61%. Similarly, in pork and buff meat samples, 27.77% and 37.17% of *S. aureus* were resistant to Gentamicin respectively. A total of nine *S. aureus* isolates were methicillin resistant and among them, five isolates were from chicken meat and two isolates each from (n=5) pork and buff meat. Out of 78 *S. aureus*, 12 (24.48%) isolates from chicken and one isolate from pork were found to be inducible clindamycin resistant. However, no any *S. aureus* isolates from buff meat were inducible clindamycin resistant (Table 3).

Table 2: Antibiotic resistance pattern of *E. coli*

Antibiotics	N (%) of resistant E. coli isolates from			
	Chicken	Buff	Pork	Total
Ciprofloxacin	16 (32.65)	0	1 (6.67)	17 (20.23)
Co-trimoxazole	37 (75.51)	1 (5)	6 (40)	44 (52.38)
Ceftazidime	3 (8.16)	1 (5)	0	4 (4.7)
Cefotaxime	6 (12.24)	6 (30)	1 (6.66)	13 (15.47)
Chloramphenicol	16 (32.65)	0	0	16 (19.04)
Tetracycline	46 (93.87)	1 (5)	6 (40.0)	53 (63.09)
Imipenem	0	0	0	0
Amikacin	3 (6.12)	3 (15)	1 (6.66)	7 (8.33)
Aztreonam	0	0	0	0
Gentamicin	9 (18.36)	0	1 (6.66)	10 (11.90)
Ampicillin	38 (77.55)	3 (15)	5 (33.33)	46 (54)
Cefepime	0	0	0	0
Piperacillin-Tazobactam	0	0	0	0

Table 3: Antibiotic resistance patterns of *S. aureus*

Antibiotics	N (%) of S. aureus isolates from			
	Chicken	Buff	Pork	Total
Cefoxitin	5 (11.90)	2 (11.11)	2 (11.11)	9 (11.5)
Linezolid	0	0	0	0
Co-trimoxazole	2 (4.76)	1 (5.55)	0	3 (3.84)
Tetracycline	20 (47.61)	0	1 (5.55)	21 (26.92)
Gentamicin	19 (45.23)	5 (27.77)	5 (27.77)	29 (37.17)
Clindamycin	3 (7.14)	0	0	3 (3.84)
Ciprofloxacin	22 (52.38)	3 (16.66)	2 (11.11)	27 (34.61)
Erythromycin	9 (21.42)	1 (5.55)	4 (22.22)	14 (17.94)

Among *E. coli* isolates, 51.19% were MDR and most of them were isolated from chicken meat. Similarly, 23.07% of *S. aureus* were MDR and most of them were contributed by chicken meat. However, the numbers of MDR isolates were lower in buff meat samples (Table 4).

Table 4: Distribution of multi-drug resistant bacterial isolates

Sources	Chicken		Buff		Pork		
Bacteria	Number of isolates	MDR N (%)	Number of isolates	MDR N (%)	Number of isolates	MDR N (%)	Total MDR N (%)
E. coli	49	36 (73.47)	20	2 (10)	15	5 (33.33)	43 (51.19)
S.aureus	42	17 (40.48)	18	0	18	1 (5.55)	18 (23.07)

4. Discussion

This study showed that the higher presence of *E. coli* was found in the buff sample followed by chicken and pork. Likewise, the higher presence of the *S. aureus* was found equally in both pork and buff followed by chicken (70%). This result contradicted with previous study conducted in Nepal, Washington DC and Nigeria. Study conducted in Nepal showed the prevalence of *E. coli* was found to be 66.6% and 40% in chicken and buffalo meat, respectively (Bantawa *et al*. 2018). Research in Washington DC, reported that out of 210 collected sample, *E. coli* was found to be 38.7% in chicken, 19% in beef and 16% in pork (Nathan & Cars 2014). A study performed in meats sample in Nigeria, reported that *S. aureus* was found to be 80% in chicken, 28% in beef & goat and 64% in pork (Zhao *et al*. 2001). This variation in prevalence may be due to factor of hygiene, sanitization, difference in the quality of sample, errors during sample processing, difference in the geographical conditions and the use of different techniques and interpretation guidelines.

In the present study, *E. coli* was the most commonly detected bacteria in comparisons to the S. aureus. A similar finding was reported from Kathmandu, where they found more than 80.0% of sample had coliform (Maharjan *et al*. 2006). Previous study conducted in Nepal showed the prevalence of *E. coli* was found to be 66.6% and 40.0% in chicken and buffalo meat, respectively (Bantawa *et al*. 2018). In this study, out of 84 isolates of *E. coli* from all three meat samples, 43 showed MDR pattern. In comparison to buff, pork and chicken meat, a higher number of MDR strains were found in chicken meat sample. From chicken meat, 74.47% MDR strains were isolated whereas 33.33% and 10% were found in pork and buff meat respectively. In poultry, it was reported that intestinal microflora changed into MDR, 77.4% from Saudi Arabia (Al-Ghamdi *et al*. 1999), 81.3% from households and small-scale farms in Vietnam (Nguyen *et al*. 2015). A study conducted in chicken breast sample in the United States showed 83.5% prevalence of *E. coli*, of which 38.9% isolates were MDR (Zhao *et al*. 2012). The variation on the rate of resistance can be related to the difference in time and place and depends on the amount of antibiotics used. Another reason for the difference in resistance rates might be a rapid change in antibiotic sensitivity patterns of bacteria within a short period.

S. aureus isolates, which were resistant to Cefoxitin (30 mcg), were considered as methicillin resistant. In our study, 9 MRSA strain were detected among which higher MRSA strain were found in chicken meat (n=5) whereas in pork and buff meat (n=2, each). MRSA has been reported in a variety of meats including raw chicken, pork, veal, beef and mutton. Our finding coincides with the MRSA results from different countries. (De Boer *et al*. 2009) reported prevalence of MRSA was highest in chicken (16.0%) followed by veal (15.2%), pork (10.7%) and beef (10.6%). Similar study performed on meat sample in Netherlands reported that out of 2217 sample of meat 264 (11.9%) of MRSA were isolated among which 16.0% and 10.7% were from chicken and pork meat respectively (van Loo *et al*. 2007). MRSA in chicken meat was found to be 20.0% in Bangladesh (Ali *et al*. 2017).

5. Conclusion

This research sheds light on the presence of multidrug-resistant *E. coli* and *S. aureus* in chicken, buffalo, and pork meat sold in the Kathmandu Valley. The findings highlight the serious threat to public health presented by these microorganisms in commercially available raw meat. Future research endeavors should delve deeper into the molecular mechanisms and genetic factors driving antimicrobial resistance in *E. coli* and *S. aureus* found in meat samples. Longitudinal studies across different seasons and regions can provide a more comprehensive understanding of the prevalence, distribution, and dynamics of drug-resistant bacteria, enabling evidence-based interventions to combat this pressing issue. These efforts will aid in effectively addressing potential threats. Additionally, conducting training and awareness programs is necessary to decrease the indiscriminate use of antimicrobials and thereby mitigate the development of drug resistance in poultry and livestock.

References

Ahmad, R. S., A. Imran, and M. B. Hussain, 2018. Nutritional composition of meat. Meat science and nutrition 61:10.5772
DOI: 10.5772/intechopen.77045

Al- Ghamdi, M. S., F. El- Morsy, Z. H. Al- Mustafa, M. Al- Ramadhan, and M. Hanif, 1999. Antibiotic resistance of Escherichia coli isolated from poultry workers, patients and chicken in the eastern province of Saudi Arabia. Tropical Medicine & International Health 4(4):278-283.
DOI: 10.1046/j.1365-3156.1999.00392.x
PMID:10320654

Ali, Y., M. A. Islam, N. H. Muzahid, M. O. F. Sikder, M. A. Hossain, and L. W. Marzan, 2017. Characterization, prevalence and antibiogram study of Staphylococcus aureus in poultry. Asian Pac. J. Trop. Biomed 7(3):253-256.
DOI: 10.1016/j.apjtb.2016.12.001

Bantawa, K., N. Sah, D. Limbu. P. Subba, and A. Ghimire, 2019. Antibiotic resistance patterns of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, *Shigella* and *Vibrio* isolated from chicken, pork, buffalo and goat meat in eastern Nepal. BMC research notes 12(1). 1-6.
DOI: 10.1186/s13104-019-4798-7
PMID: 31752992 PMCID: PMC6873459

Bersisa, A., D. Tulu, and C. Negera, 2019. Investigation of bacteriological quality of meat from abattoir and butcher shops in Bishoftu, Central Ethiopia. Int. J. Microbiol. 2019.
DOI: 10.1155/2019/6416803
PMID: 31191657 PMCID: PMC6525944

De Boer, E., J. T. M. Zwartkruis-Nahuis, B. Wit, X. W. Huijsdens, A. J. De Neeling, T. Bosch, and A. E. Heuvelink, 2009. Prevalence of methicillin-resistant Staphylococcus aureus in meat. Int. J. Food. Microbiol 134(1-2):52-56.
DOI: 10.1016/j.ijfoodmicro.2008.12.007
PMID:19144432

Shrestha, A., A. M. Bajracharya, H. Subedi, R. S. Turha, S. Kafle, S. Sharma, and D. K. Chaudhary, 2017. Multi-drug resistance and extended spectrum beta lactamase producing Gram negative bacteria from chicken meat in Bharatpur Metropolitan, Nepal. BMC research notes 10(1):1-5.
DOI: 10.1186/s13104-017-2917-x
PMID: 29116010 PMCID: PMC5678746

Marshall, B. M., and S. B. Levy, 2011. Food animals and antimicrobials: impacts on human health. Clinical microbiology reviews 24(4):718-733.
DOI: 10.1128/CMR.00002-11
PMID:21976606 PMCID:PMC3194830

Maharjan, M., V. Joshi, D. D. Joshi, and P. Manandhar, 2006. Prevalence of Salmonella species in various raw meat samples of a local market in Kathmandu. Annals of the New York Academy of Sciences 1081(1):249-256.
DOI: 10.1196/annals.1373.031
PMID:17135520

Nathan, C., and O. Cars, 2014. Antibiotic resistance problems, progress, and prospects. N. Engl. J. Med 371(19):1761-1763.
DOI: 10.1056/NEJMp1408040
PMID:25271470

Nguyen, V. T., J. J. Carrique-Mas, T. H. Ngo, H. M. Ho, T. T. Ha, J. I. Campbell, and C. Schultsz, 2015. Prevalence and risk factors for carriage of antimicrobial-resistant Escherichia coli on household and small-scale chicken farms in the Mekong Delta of Vietnam. J. Antimicrob. Chemother 70(7):2144-2152.
DOI: 10.1093/jac/dkv053
PMID: 25755000 PMCID: PMC4472326

Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, and J. Meng, 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC, area. Applied and Environmental Microbiology 67(12): 5431-5436.
DOI: 10.1128/AEM.67.12.5431-5436.2001
PMID:11722889 PMCID:PMC93326

The Preliminary Online Survey on E-waste Literacy in Nepal

Jyoti Giri[1,2,3*], Hemraj Joshi[1], Geeta Aryal[1], Cynthiya Shrestha[1] and Rameshwar Adhikari[2,4]

[1]Tri-Chandra Multiple Campus (TC), Tribhuvan University (TU), Ghantaghar, Kathmandu, Nepal
[2]Nepal Polymer Institute (NPI), Kathmandu, Nepal
[3]Nepal Development and Research Institute (NDRI), Manbhawan, Lalitpur, Nepal
[4]Research Centre for Applied Science and Technology (RECAST), Tribhuvan University (TU), Kirtipur, Nepal

***CORRESPONDING AUTHOR:**
Jyoti Giri
Email: girijys@yahoo.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI : https://doi.org/10.3126/njst.v21i2.67161

Date of Submission: Sep 6, 2022

Date of Acceptance: Jan 30, 2023

ABSTRACT

Electrical and electronic wastes (E-wastes) are complex waste and a new to the community. They are boon of new advanced technology serving the sophisticated society on the Earth; however, they are disaster from environmental point of view. E-waste is complex in its composition with small quantities of expensive metals such as Gold, Silver, Platinum, Copper and even toxic metals such as Lead, Cadmium, and rare earth metals (including Yttrium, Indium and many more). Knowing the value of these metals and their recyclability as well as the toxicity of some constituents, it has become important to develop proper regulations as well as appropriate strategies and technologies to extract and detoxify all the elements present in them. Moreover, that one should know the type of waste he or she is producing and be responsible for its proper management. With these concerns, from September 6 - Oct 6, 2020 during Covid-19 lockdown period, an online survey was conducted by creating a Google form in which 453 participants submitted their opinion responding to the queries prepared in the form. The survey was requested by sending group E-mails and the virtual platform of Facebook, messenger with tagging many people in academia, waste management, stakeholders of science waste workers and many other people in community for wide dissemination of survey. During the pandemic lockdown the whole world was subjected to virtual platform. Education sector came first in the virtual platform during lockdown and the data response show that women from education background are in majority due to their free time waiting for virtual class. The survey showed ratio of women respondents higher than men, (with women to men ratio of 1.6:1 and 76 % were students). The higher numbers of replier were from the age group of 17-40. The replies indicated that 63% of reporters were aware of the E-wastes. However, 83% of people know the health hazard of E-waste. The interesting part of the survey was that 73.7% of respondents were segregating E-waste from other wastes due to their economic value. As 75% reported their knowledge about precious metal contents in it and 80.6% knew their recyclability. The survey revealed that a significant fraction of the population, especially comprising the students, was literate on E-wastes knowing about their economic values and possible hazards to health and the environment.

Keywords: Electrical and electronic equipment (EEE), E-waste, International policies, Online survey, Nepal

1. Introduction

Electronic wastes (E-wastes) are non-functional Electrical and Electronic Equipment's (EEE), such as computers, laptops, mobiles, handsets, televisions, refrigerators, and other appliances which have been dumped by users at the end of their economic value and cannot be reused further (Giri & Adhikari 2020). The European Union Directive 2012/12/19/EU refers E-waste as Waste of Electric and Electronic Equipment (WEEE) (Directive 2002/96/EC 2003; Directive 2012/19/EU 2012). This WEEE are classified as a special waste (13.030 wastes) in the International Classification of Standard's (ICS) catalog 13.030.3, along with nuclear waste, hospital waste, and other hazardous waste by International Organization for Standardization (ISO). Due to rapid technical innovations, planned obsolescence in the electronics sector and rising consumer demand for new electronic devices, E-waste is the fastest over growing waste on the planet. The yearly introduction of new version of smartphones, computers, and other electronic gadgets, results in the accumulation of unutilized older versions. E-waste is fast increasing due to the shorter invention periods and poor recycling levels (Perkins *et al.* 2014) Electronic items make life easier and have become a status symbol, leading to an increase in electronic trash. Furthermore, as technology advances, the price of electronic devices fall and become more accessible, allowing people to dispose of their old gadgets with ease (Okwu & Onyeje 2014). In addition to that, manufacturers' purposeful impermanence of their EEE by continuous releasing new models with greater features persuades customers to believe that purchasing new gadgets is often cheaper and more convenient than upgrading current ones. The liquid crystal display (LCD), displays, easily replaced cathode-ray tube (CRT) monitors which are smaller and consume less energy, and is the best example of this trend, resulting in a significant CRT monitor disposal. Similarly, aggressive marketing and enticing incentives by the manufacturers, lure people to seek out new EEEs.

The vast majority of the EEE are made up of the two primary units: i) polymer, and ii) the inner complex metallic setting, sensor coated printed circuit board (PCB) which consist of the variety of harmful and non-harmful inorganic such as expensive gold (Au), silver (Ag), Copper (Cu) and Palladium (Pd)), toxic and heavy metals such as Nickel (Ni), Tin (Sn), Lead (Pb), Chromium (Cr), and iron (Fe) and organic components such as polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and brominated flame retardants (BFR) (Needhidasan et al. 2014). Lead and Cadmium (Cd), in circuit boards and mercury (Hg) in switches, are extremely poisonous causing major health problems in the central nervous system, blood circulation system, excretory organs, and brain growth, etc (Rawat *et al.* 2020).

One of the study shows, E-waste causes, physical, mental, and neurological retardation (Grant *et al.* 2013). Copper being a flame retardant metal acts as a catalyst for dioxin formation when burned (Cui & Zhang 2008). It has been noticed that thyroid and lungs functioning, reproductive health, growth, and changes in physiochemical activities in cell are harmed by exposure to E-waste (Grant *et al.* 2013; Beiyuan *et al.* 2016). Contrary to this, EEE is inextricably linked to global economic growth. Increased discretionary incomes, urbanization, mobility, and industrialization in various regions of the world are contributing to rise EEE consumption. One of the data showed, the average intake of the overall weight (excluding photovoltaic panels) of global EEE increases by 2.5 million metric tons (Mt) per year (Forti *et al.* 2020) which ultimately converts to WEEE.

Asia produced highest amount of the E-waste in 2019, (24.9 Mt), followed by the Americas (13.1 Mt) and Europe (12 Mt). In term of per capita, Europe ranked first then Oceania (Forti *et al.* 2020). The management part of collection and recycling is 42.5% done by Europe in 2019, later followed Asia.

During covid-19 lockdown 1.3 billion students started online class and 300 millions people started working from home in the virtual plate using EEE which after 10 years anticipated to convert to WEEE (Li & Lalani 2020; International Labor Organization 2020; International Data Corporation 2020). Thereafter, the amount of WEEE will increase by 3% per year which is higher than the world wide population growth (Yu *et al.* 2020).

The time has come to implement correct E-waste management policies as these E-wastes are being illegally traded and dumped which cost approximately $19 billions every year (UNEP 2015). The agreement in Basel convention and Basel Ban are not being regulated by the signatory countries, however, developed countries do not allow E-waste to enter into the normal trash (Li *et al.* 2013). It goes to recycling sectors which focus on labor intensive process and cause severe complications in health and the environment of the local

territory (Han *et al.* 2018; Dias *et al*. 2019; Pariatamby & Victor 2013).

The developed countries such as Germany, Japan, Europe, China have implemented strict policies of E-waste management such as Extended Producer Responsibility (EPR), Polluters Pay, per-purchase tax payment, many laws and principles of 3R's, 7R's which suits their EEE business circular economy (the ElektroG) (Wang *et al.* 2017; Sander *et al.* 2007; Walther *et al.* 2010; Bhutta *et al.* 2011; Otmar 2014). At the same moments China refused foreign trash as it has been the world's largest junkyard since 2014 (Zhang 2020; Li *et al.* 2006; Pariatamby & Victor 2013; Tong & Yan 2013; *Wang et al.* 2017). In case of southern Asia, India become the first to implement EPR based E-waste management which came in action on 1st May 2012 (PACE Nepal Pvt. Ltd., 2017; Suja *et al.* 2014; Rathore *et al.* 2011; Khetriwal *et al.* 2019).

Nepal is importer of EEE, from neighboring countries and third world countries. According to Global E-waste monitor in 2020, Nepal had accumulated 28 kilo tonns of E-waste in 2019 i.e. 0.9 Kg per inhabitant (Forti et al. 2020). Nepal Telecommunication Authority (NTA) had also collected the cellphone import data from Tribhuvan International Airport (TIA) custom department in early 7 months in 2075/76 BC fiscal year which was 2,686,648 pieces which is many times higher than the earlier fiscal year 2074/75 (Nepal Telecommunication Authority 2021; Aryal 2020). It clearly indicates the ratio of E-waste production in near future in Nepal (Awale 2018; PACE Nepal Pvt. Ltd. 2017).

Article 30 of Nepal's 2015 constitution establishes a legal right to live in a clean environment and right to compensate for damage caused by environment degradation. In this scenario, Nepal is also showing concern to protect environment from the waste and regulate the solid waste management Act in 2011, however, there is no mention for E-waste management. E-waste is the complex waste which needs to be handled differently and for that, skilled hands are necessary (Nepal Telecommunication Authority 2017; Mandal 2021; Satyal 2017, Parajuli *et al.* 2018; Turaga *et al.* 2019).

As EEE are the high-tech gadgets and from the stage of its manufacture to its end use and its waste management require high level of knowledge and understanding. Therefore, this survey tries to explain the literacy of the EEE users specially the youth who started their online teaching learning program and work from home during lockdown period of covid-19 pandemic.

2. Survey Objective and Methodology

2.1 Online Survey

The status and effects of E-waste in Nepal and the analysis of the awareness concerning E-waste management, a short pilot online survey was conducted using Google form. The survey was run for about a month, Sept 6- Oct 6, 2020, the time of the global crisis "COVID-19 pandemic" during lockdown period. Physical separation and quarantine were required to mitigate the impacts of the COVID-19 epidemic. To comply with this demand, different aspects of human activity (such as purchasing, learning, working, meeting, and entertaining) have transferred to online, resulting in a faster adoption of developing digital technologies by the general public. Electronics like televisions, computers, laptops, and cellphones have been increasingly popular, particularly during this epidemic. Looking at the pace of the technological advances, it did not take much longer for these devices to be discarded and replaced with their newer versions.

In the survey, pre-designed questionnaires were set targeting E-gadget users. The main objective was to collect information on the majority usage of Electrical and Electronic Equipment (EEE) by certain age groups, professions, and the ratio of family members. In addition to that, the questionnaires also revealed the habits of users regarding the disposal or storage of E-waste. The survey focused on the respondent's quantity of electronic devices, their purpose for using them, their awareness of recycling trends, practices employed for managing their E-waste, and their satisfaction with E-waste handling.

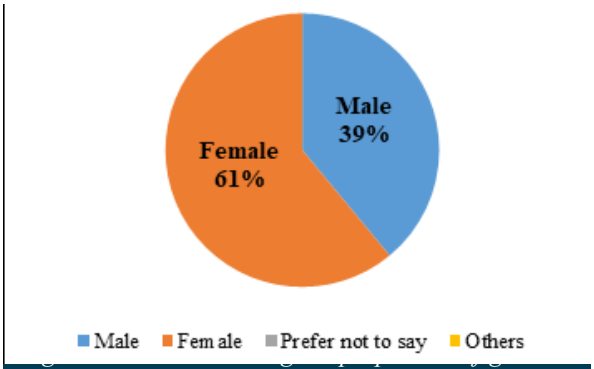
[Google form link: https://docs.google.com/forms/d/e/1FAIpQLSepAnlL5ifImgmiq6HjAlkqgsIky5h3hPBcRoj5k18C_MKU5A/viewform]

2.1.1 Respondents

In all, 453 people took part in the survey, representing various age groups and occupational backgrounds.

2.1.2 Male / Female ratio

This survey received responses from 176 men and 276 women, with a male participation rate of 39% and a female participation rate of 61% as shown in the pie chart [Fig. 1].



who responded to the survey

2.1.3 Age bars

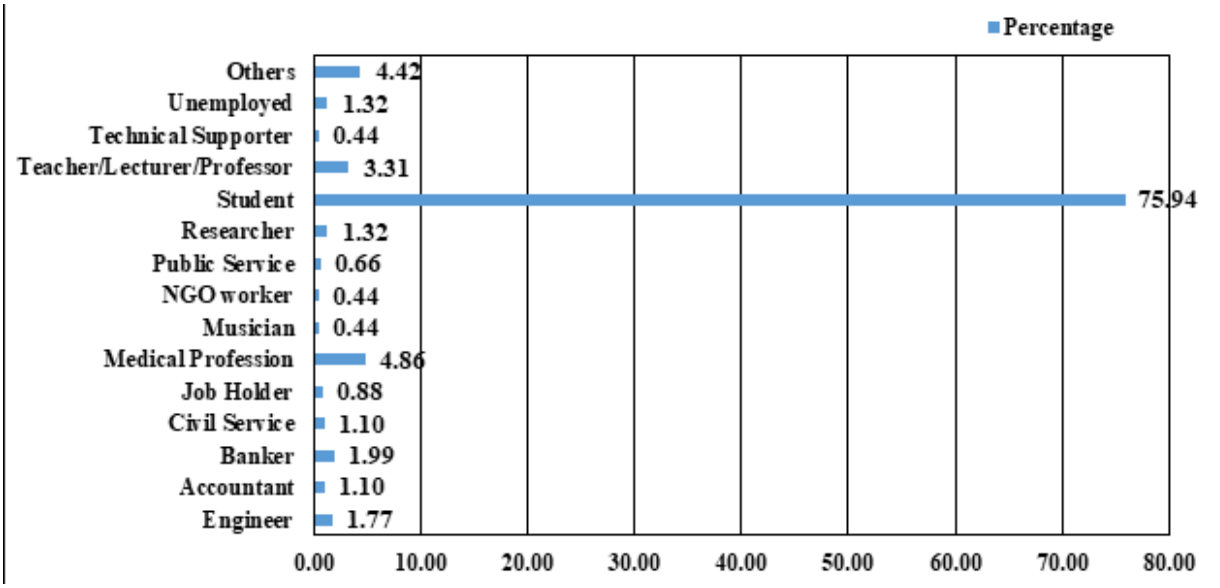
The respondents were classified into different sub-groups according to their age as shown in Table 1. Among the total 227 respondents, the age group 17-22 had the largest participation, followed by the age group 23-40, with 221 respondents. These age groups are considered to be most familiar with the usage and application of E-gadgets.

Table1: Division of respondents into various sub-groups as per their age

Age Group (in years)	1-16	17-22	23-40	41-50	51-60	Total
Responses	1	227	221	2	2	**453**

2.1.4 Profession

This poll drew a wide range of responses based on the occupation of the respondents as shown in (Fig. 4). Students accounted for the bulk of replies (about 76%), followed by medical professionals (5%), teachers/lecturers/professors (4%), and others (4%).



2.2 Literacy on E-waste

2.2.1 Male /Female ratio

The ratio of female to male respondents in this survey is about 1.6:1. This indicates that the use of E-gadgets is more popular among the female population here in Nepal. In Nepalese society, females are more active in domestic activities justifying the higher usage of electrical equipment like microwaves, ovens, freezers, vacuum cleaners, and other similar technologies for a more convenient living (Matinga *et al.* 2019).

2.2.2 Age bars

Table 1 indicates that the age group from 17-22, which accounts for about half of all respondents, uses more E-gadgets than the other age groups. This age group often includes students who have taken the SEE and are looking for high schools, as well as those who are currently enrolled in colleges or institutions. The age group from 23-40 falls second on the list and in general consists of graduate students who are in search of employment or higher education. According to the National Population and Housing Census of Nepal 2011 report, the population of age group 15-24 years is 21.86 % (male 3,176,158 / female 3,169,721) and 25-54 years is 35.99% (male 4,707,264 / female 5,740,985) with a median age of 21.6 years (Central Bureau of Statistics Nepal 2012). These statistics reveal that the younger generation makes up a higher share of the Nepalese population and is more familiar with electronic devices than other age groups, which is supported by the highest number of participants from this age group in this survey.

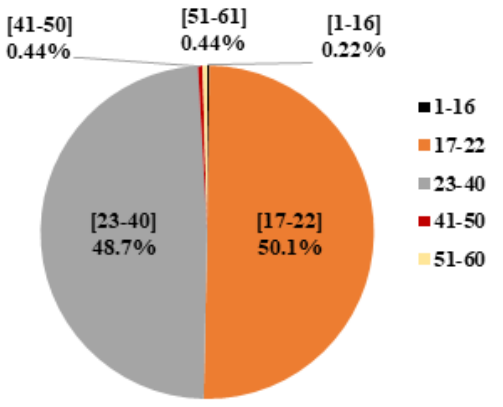


Fig. 3: Participation of different age groups in the survey in terms of percentage

2.2.3 Profession

As shown in Fig. 2, the use of E-gadgets is highest among the students, which resembles the data in Table 1. The age group from 17-22, in general, constitutes college or university graduate students and are more likely exposed to different EEEs like laptops, desktops, smart-phones, printers, etc. for their academic commitment or social media usage. The number of internet users climbed to 10.78 million in January 2021, according to national statistics. Nepal's internet users increased by 567 thousand (+5.5%) between January 2020 and January 2021, with 36.7% internet penetration in January 2021 (Data Reportal 2021). This was during the covid-19 pandemic which directly affected the teaching-learning activities of nearly 8,796,624 students as estimated by UNESCO. Most schools and colleges opted for online platforms which might be one of the reasons for maximum usage of E-gadgets by the students (Pal *et al.* 2021)

In this survey, medical professionals are the second-highest consumers of E-gadgets. Since the survey was conducted during the COVID-19 pandemic, health workers and medicinal professionals were more exposed to the use of electrical equipment like ventilators, breathing systems, and other life support equipment (GlobeNewswire 2021).

Professors, teachers, and lecturers are the third most frequent users of E-gadgets. Since the survey was conducted during the commotion of the COVID-19 pandemic, the reasons for the results are quite evident with the whole education system undergoing digitization. It has been reported that by the end of 2020, every student took at least one online class and a professional attended at least one virtual training (Mishra *et al.* 2020). This certified the increase in the use of E-gadgets among consumers.

2.3.1 Consumption pattern

According to the annual household survey 2015-16, the average household size is 4.6 persons per family in Nepal which is 4.2 in urban and 4.8 in rural (Central Bureau of Satistics Nepal 2017). Given that at least one family member has access to certain E-gadgets, the poll included a question on the number of family members accessing any kind of E-gadget. There were a total of 2217 family members registered in our survey, based on 453 respondents considered each responder belongs to a separate family. This concluded that the average household size is 4.89, which is almost similar to data

published by the annual household survey 2015-16 or we can analyze this data in another way that there is a certain increment in the average number of family members in Nepal from 2016 to 2020. So, at minimum, there is an average of 5 members per family who get access to at least one E-gadget. The total number of households in Nepal as per the population census of 2011 is 5,427,302. By prediction, at least 27,136,510 E-gadgets are operating within these families, excluding offices, business sectors, schools, etc. The average size of family predicts the total number of users who are dependent on different E-gadgets in their daily life.

2.3.2 Type of Electronic Devices in practice

The study also aimed at finding the most widely used E-gadgets, such as desktops, laptops, televisions, mobile / smart-phones, refrigerators, printers, microwaves, and other electric home appliances. Table 2 shows the data of some commonly used electronic devices in our daily life. According to the observed data, cellphones/gadgets are the most commonly used electronic equipment, accounting for about 16% of all respondents (excluding basic E-gadgets such as electric bulbs, fans, and so on). A total of 1636 mobile phones are in use, out of 453 respondents which is about an average of 3.6 (~4) mobile phones per person. Besides that, the other gadgets in use are laptops 646 (6%), televisions 513 (5%), refrigerators 440 (4%), desktops 213 (2%), etc., among 453 respondents. A whopping total of 10,038 electronic devices were recorded from 453 respondents and the ratio of total E-gadgets in use to total respondents is 22:1 i.e. 1 respondent holds 22 E-gadgets.

Table 2: The data showing the number and types of E-gadgets used by 453 respondents

E-waste	Number	E-waste	Number
Electric Bulb	2256	Television	513
Cellphone/Gadgets	1636	Refrigerator	440
Electric Fan	1292	Water Pump	437
Earphone/Headphone	903	Desktop	213
Other	722	Vacuum Cleaner	181
Laptop	646	Printer	134
Wi-Fi Router	549	Microwave	116
		Total	**10,038**

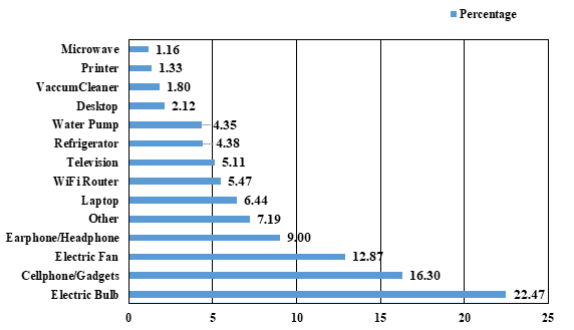


Fig. 4: The chart showing different types and percentages of the E-gadgets currently in use by the respondents

2.3.3 Replacing Practices of E-gadgets

In Nepal, there is a strong demand for the import of E-gadgets and cellphones. The import of more short-lived E-gadgets from foreign countries is also one of the major reasons for the increase in the number of E-waste. Due to the low economic status of the majority of Nepalese people, E-gadgets merchants and businessmen focus on importing low-cost, low-quality electronic goods with short lifespans, contributing to a high amount of E-waste production (Khatri 2019). The lifespan of many electronic equipment have been shortened by appealing new designs, marketing, and compatibility concerns. In the previous decade, the typical lifespan of a new computer has fallen from 6 to 2 years (Bhutta *et al.* 2011) the global market of electrical and electronic equipment (EEE). Cell phone companies design phones that aren't built to survive long, forcing consumers to buy new phones more frequently (Lamichhane 2017).

In general, a total of 1299 cellphones were replaced by 453 respondents within 5 years, as per the survey. In five years, about 6.4% of all respondents changed five cell phones, 8.6% replaced four cell phones, 28.03% replaced three cellphones, 37.74% of all respondents changed two smartphones, and 12.14% replaced at least one cellphone. On average, 2.87(~3) cellphones were replaced by each respondent within the 5 years duration. That means, the average number of cellphones as E-waste produced per person within 5 years of interval is 3.

2.3.4 Budget/ Expenses in E-Gadgets

According to our findings, each person annually

spends about NRs. 33,946.00 on E-gadgets. The annual expenditure by 453 respondents on E-gadgets is about NRs. 15,377,745.00. This indicates increase in the inclination of Nepalese people in buying new, sophisticated, and updated versions of smart E-gadgets.

2.4 E-waste generated

The E-waste generated per family was calculated by dividing the total number of generated E-waste by the total number of respondents recorded in this survey, considering each responder belong to a different family. The total sample size of 453 respondents, gave an average E-waste generation per family. In these 453 families, there was a total of 4781 pounds of E-waste generated. There are 2,217 people in total in 453 households. As per the calculation, every single person in this survey is responsible for converting every 3 E-gadgets into E-waste. Out of the total E-waste (4781), 1020 are electric bulbs (21%), 881 are cellphones (18%), 777 are headphones (16%), 432 are electric fans (9%), 227 are televisions (5%), and 461 are other forms of E-gadgets (10%). Table 3 shows the number of various E-gadgets reported in the survey.

Table 3: The different types and numbers of E-waste reported by the respondents in the survey

E-waste	Number	E-waste	Number
Television	227	Microwave	51
Cellphone/Gadgets	881	Electric Bulb	1020
Laptop	188	Electric Fan	432
Desktop	146	Water Pump	158
Printer	63	Wi-Fi Router	184
Refrigerator	121	Earphone/ Headphone	777
Vacuum Cleaner	72	Other	461
		Total	4,781

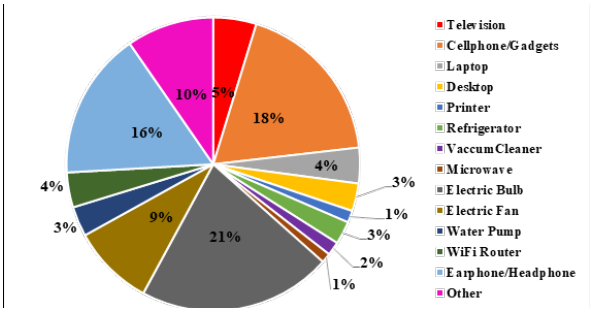


Fig. 5: The chart showing percent composition of different types of E-waste reported during the survey

2.5 Practices/ Responsibility

2.5.1 Practices

As per the survey, about 63.4% of total respondents were familiar with E-waste formerly, and the remaining 36.6% were unknown of E-waste. Around 83.1% of respondents knew about the consequences of E-waste on the environment and health, and roughly 78.7% were knowledgeable about the toxic substances included in E-waste, which is quite impressive. However, just 73.7% of all respondents separated their E-waste from other trash.

In terms of the recycling process, approximately 80.6% of the respondents were aware that E-waste may be recycled and refurbished. Moreover, around 85.9% were familiar with the 4R (reuse, reduce, recycle, redesign) process and around 75.1% of the respondents were aware that E-waste contains valuable metals such as gold, silver, copper, and many more.

Fig. 6 demonstrates that 45.5% of all respondents sell their E-waste to scavengers, while the remaining 5.5% sell it to scrap hawkers. About 33.8% of all respondents dispose their E-waste in municipal trash, 40.6% just keep it at home, 14.1% throw it away, and 7.1% burn it. About 15.9% of total respondents recycle their E-waste through E-waste recycling companies while 0.2% recycles by themselves.

In Nepal, there are many E-waste handlers such as Solid Waste Management Association Nepal (SWMAN), Blue Waste to Value Pvt. Ltd (BW2V)., Action Waste Pvt. Ltd., Creasion Nepal, Purano Kagaj, Fohar Malai, Kagaj Kabari, DOKO Recyclers, etc.

For our question, “Do you know any E-waste recycling company in Nepal?” 19.9% knew only about Khalisisi while 13.2% knew only about DOKO Recyclers and 18.3% knew about both the companies. Besides, only 0.4% knew about BW2V and 0.2% knew about Creasion Nepal.

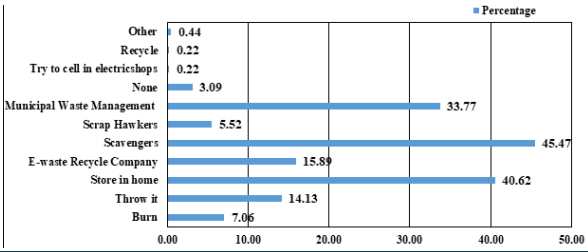


Fig. 6: The chart showing different types of E-waste management practices as reported by the respondents in the survey

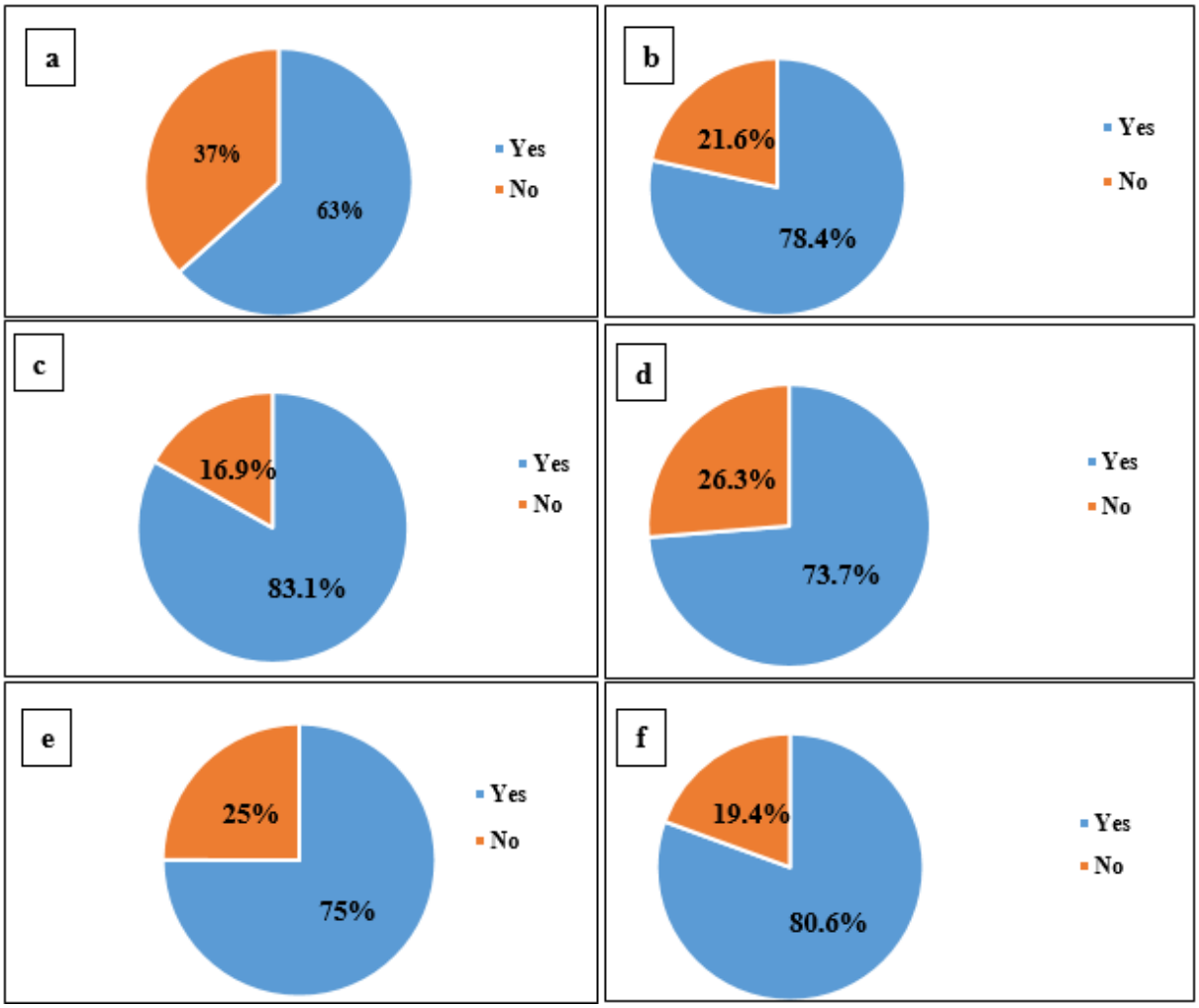


Fig.7. Percentage of respondents for the question; Do you a) know E-waste? b) know about hazardous elements in E-waste? c) know the health and environmental impacts of E-waste? d) separate E-waste from other waste? e) know E-waste contains precious metals? f) know E-waste can be recycled?

Fig. 7 gives the ratio of awareness regarding E-waste in the community which directly correlates with education level of consumers. As highly educated-Elite person used many EEE to amplify their working ability quantitatively as well as qualitatively who are also one of the major groups producing E-waste and as international policy of Extended Producer Responsibility (EPR) states that producer and their knowledge to E-waste will guide them to do proper E-waste management (Wang *et al.* 2017). In this survey all of the respondents are well educated in their respective subjects who are supposed to be responsible citizens of Nepal.

2.5.2 Responsibility

According to the Fig. 7, the majority of respondents were aware of E-waste, its consequences, and its economic value, but were unsatisfied with E-waste management in Nepal.

The survey came to show 24.9% of respondents claiming the government's responsibility for the proper management of E-waste but on the other side, 41.9% believed that the consumers were equally answerable. Moreover, 17.7% believed that manufacturing companies are liable while the remaining 6.6% argued that NGOs/INGOs are responsible for the E-waste management. But, in reality, all these sectors including consumers are responsible for this most challenging

issue which is agreed by 5.6% of the respondents.

2.6 Challenges of E-waste management in Nepal

To ensure proper management of the E-waste in Nepal, there should be a predefined model for the effective collection and disposal of the E-waste generated. Along with it, appropriate coordination with the local informal bodies is the most. Although certain measures and guidelines have been highlighted here in Nepal, several challenges need to be tackled for acquiring a sustainable E-waste management system. Some of the measures to tackle E-waste in Nepal are listed below

- The first step is to quantify all the electronic products that are being sold in the Nepalese market which can be achieved with the help of the Department of Customs (DoC) and Trade and Export Promotion Centre (TEPC). Along with that, it is important to document all the E-gadgets that enter Nepal *via* migrant workers to track the exact amount of electronic goods being circulated (Parajuly *et al.* 2018).
- An extensive research regarding the consumption and the usage patterns of the electronics among different groups of consumers shall help in getting comprehensive data about the E-waste generated.
- Implementing potent legislative guidelines and policies for the better management of E-waste is another crucial step in the management of E-waste. However, Nepal has not yet built its act and policy implemented for E-waste management.
- The informal recycling practices and bodies are of great concern in handling the E-waste across the globe. Without the strict guidelines and rules, the recognition of such informal practices here in Nepal is much more crucial (Khatri 2019). The majority of the waste collectors come from an underprivileged background which makes them more susceptible to health risks. Aside from that, most recyclers are victims of pay protection money, a disorganized trade and transportation system, and a risky business environment owing to the lack of strong regulatory standards in this industry. (Bajracharya 2022 August 22 onlinekhabar)
- Finally, there appears to be a lack of self-awareness among suppliers and consumers about the relevance of E-waste management and the necessity for an updated E-waste management system. So, all these learnings must be taken into consideration before building a resource-oriented sustainable waste management system here in Nepal (Parajuly et al. 2018).

3. Conclusion

E-wastes are hazardous to health and the environment, non-biodegradable, and accumulate in the environment, generating a global threat in garbage volume. E-waste belongs to a special type of garbage, and its management cannot completely rely on traditional and outdated methods; instead, it necessitates the use of cutting-edge of technologies. The COVID-19 epidemic has indeed exacerbated the problem of E-waste management even more. Industrial countries have cutting-edge waste management facilities, finances, and technologies, but it has been found that most of their electronic garbage is not recycled and instead exported to developing countries with laxer environmental regulations. Nepal, on the other hand, lacks the requisite technology for E-waste treatment, and with India's prohibition on E-waste imports, it would be possible that we have to deal with the worst E-waste management disaster in its history.

The present research was aimed to study the preliminary E-waste status and awareness in Nepal who are using E-gadgets (smart phone) frequently. It has been found that among 453 respondents, 61% of the respondents were females, implying that E-gadgets are more popular among Nepalese females. Likewise, the respondents belonging to the age group from 17-22 constitute the highest number of E-gadgets users (50.1%). Students are the most frequent users of E-gadgets accounting for 75.94% of all respondents. As per the survey, the average family size was 4.89 and every 1 out of 453 respondents owned twenty-two E-gadgets. In five years, each survey responder changed an average of three cell phones. The most commonly used E-gadget and the most discarded E-waste are operational and malfunctioning electric lamps. 63.4% of the total respondents were well acquainted with the term E-waste while, 78.7% were well aware about the hazardous elements present in E-waste and 83.1% were well informed about the harmful impacts associated with this E-waste. Even though 85.9% of respondents were conversant with 3R, only 73.7% of all respondents separated E-waste from other trash. 45.47% of respondents dispose of their E-waste by selling it to scavengers. 75.1% of those surveyed were knowledgeable about the valuable metals found in e-waste. DOKO recyclers seem to be more popular among the respondent as compared to

other listed recycling firms in Nepal. Finally, 24.9% agreed that the government should be fully responsible for E-waste management. This E-waste recycling issue in Nepal must be addressed to prevent the adverse consequences of E-waste recycling by implementing effective collecting and futuristic disposal strategies following international principal of EPR for sustainable E-waste management.

Acknowledgement

Authors acknowledge honorable formal Science Minister Er. Ganesh Shah for his encouragement to prepare the database on E-waste status in Nepal. University Grant Commission (UGC) for providing mini research grant on E-waste SRDIG-78/79-SRDIG7817es&T-07.

References

Awale, S., 2018. What will Nepal do with its e-waste?As purchasing power rises, Kathmandu faces the problem of electronic waste disposal. *Nepali Times*.

Awale, S., 2018. What will Nepal do with its e-waste?As purchasing power rises, Kathmandu faces the problem of electronic waste disposal. Nepali Times.

Bajracharya, N., 2022. E-waste in Kathmandu is a growing problem, and here is a small effort to address this. Onlinekhabar, 2022, August 22.

Beiyuan, J., D. C. W. Tsang, Y. S. Ok, W. Zhang, X. Yang, K. Baek, and X. D. Li, 2016. Integrating edds-enhanced washing with low-cost stabilization of metal-contaminated soil from an e-waste recycling site. *Chemosphere*, **159**, 426–432. DOI: 10.1016/j.chemosphere.2016.06.030 PMID:27337434

Bhutta, M. K. S., A. Omar, and X. Yang, 2011. Electronic waste: a growing concern in today's environment. *Economics Research International*, **2011**, 1–8. DOI: 10.1155/2011/474230

Central Bureau of Statistics Nepal., 2012. National population and housing census 2011 (national report). *Government of Nepal, National Planning Commission*, **01**.

Central Bureau of Statistics Nepal., 2017. Annual household survey nepal 2015/16. *Government of Nepal, National Planning Commission*.

Cui, J., and L. Zhang, 2008. Metallurgical recovery of metals from electronic waste: A review. *Journal of Hazardous Materials*, **158**(2–3), 228–256. DOI: 10.1016/j.jhazmat.2008.02.001 PMID:18359555

Data Reportal., 2021. *Digital 2021: Nepal*.

Dias, P., A. M. Bernardes, and N. Huda, 2019. Ensuring best e-waste recycling practices in developed countries: an Australian example. *Journal of Cleaner Production*, **209**, 846–854. DOI: 10.1016/j.jclepro.2018.10.306

Directive 2002/96/EC., 2003. Of the European parliament and of the council on waste electrical and electronic equipment (weee). *Official Journal of the European Union*.

Directive 2012/19/EU., 2012. Of the European parliament and of the council on waste electrical and electronic equipment (weee). *Official Journal of the European Union*.

Forti, V., C. P. Baldé, R. Kuehr, and G. Bel, 2020. *The Global E-waste Monitor 2020* (Issue July).

Giri, J., and R. Adhikari, 2020. Urgency of proper e-waste management plan in Nepal: an overview. *Nepal Journal of Science and Technology*, **19**(1), 107–118. DOI: 10.3126/njst.v19i1.29790

GlobeNewswire., 2021. *Medical Electronics Market with Covid-19 Impact Analysis by Component, Device Classification, Application And Region - Global Forecast to 2026*.

Grant, K., F. C. Goldizen, P. D. Sly, M. N. Brune, M. Neira, M. van den Berg, and R. E. Norman, 2013. Health consequences of exposure to e-waste: a systematic review. *The Lancet Global Health*, 1(6), e350–e361. DOI: 10.1016/S2214-109X(13)70101-3 PMID:25104600

Han, W., G. Gao, J. Geng, Y. Li, and Y. Wang, 2018. Ecological and health risks assessment and spatial distribution of residual heavy metals in the soil of an e-waste circular economy park in Tianjin, China. *Chemosphere*, 197, 325–335. DOI: 10.1016/j.chemosphere.2018.01.043 PMID:29366953

International Data Corporation., 2020. *Traditional PC Shipments Continue to Grow Amid Global Economic Slowdown, According to IDC*.

International Labor Organization., 2020. Working from home: estimating the worldwide potential. In *ILO Brief April 2020*.

Khatri, B. B., 2019. E- waste management: an emerging challenge in Nepal. *NUTA Journal*, **6**(1–2), 1–4. DOI: 10.3126/nutaj.v6i1-2.23218

Lamichhane, S., 2017. Electronic waste disposal. *Rising Nepal*.

Li, C., and F. Lalani, 2020. The COVID-19 pandemic has changed education forever. this is how. World Economic Forum.

Li, J., B. N. Lopez, L. Liu, N. Zhao, K. Yu, and L. Zheng, 2013. Regional or global weee recycling. where to go? *Waste Management*, **33**(4), 923–934. DOI: 10.1016/j.wasman.2012.11.011 PMID:23337392

Li, J., B. Tian, T. Liu, H. Liu, X. Wen, and S. Honda, 2006. Status quo of e-waste management in mainland china. *Journal of Material Cycles and Waste Management*, **8**(1), 13–20. DOI: 10.1007/s10163-005-0144-3

Mandal, C. K., 2021. Use of electronic goods continues to grow but there's no policy for its disposal. *The Kathmandu Post*.

Matinga, M. N., B. Gill, and T. Winther, 2019. Rice cookers, social media, and unruly women: disentangling electricity's gendered implications in rural nepal. *Frontiers in Energy Research*, **6**, 140. DOI: 10.3389/fenrg.2018.00140

Mishra, L., T. Gupta, and A. Shree, 2020. Online teaching-learning in higher education during lockdown period of covid-19 pandemic. *International Journal of Educational Research Open*, **1**, 100012. DOI: 10.1016/j.ijedro.2020.100012 PMID:35059663 PMCID:PMC7832355

Needhidasan, S., M. Samuel, and R. Chidambaram, 2014. Electronic waste-an emerging threat to the environment of urban India. *Journal of Environmental Health Science & Engineering*, 12(36), 1–9. DOI: 10.1186/2052-336X-12-36 PMID:24444377 PMCID:PMC3908467

Nepal Telecommunication Authority., 2017. Consultation paper on regulatory framework for e-waste management. *Nepal Telecommunication Authority(NTA)*, *02*, 1–103.

Nepal Telecommunication Authority., 2021. mis report. *Nepal Telecommunication Authority(NTA)*, 198(148), 1–10.

Okwu, P. I., and I. N. Onyeje, 2014. Open access extraction of valuable substances from e-waste. *American Journal of Engineering Research*, 03(01), 299–304.

Otmar, D., 2011. e-waste management in germany. *United Nations University Institute for Sustainability and Peace (UNU-ISP)*.

PACE Nepal Pvt. Ltd., 2017. *Final Report on Inventory Preparation of E-Waste and Its Management in Kathmandu Valley*.

Pal, K. B., B. B. Basnet, R. R. Pant, K. Bishwakarma, K. Kafle, N. Dhami, M. L. Sharma, L. B. Thapa, B. Bhattarai, and Y. R. Bhatta, 2021. Education system of Nepal: impacts and future perspectives of COVID-19 pandemic. *Heliyon*, 7(9). DOI: 10.1016/j.heliyon.2021.e08014 PMID: 34568606 PMCID: PMC8455144

Parajuly, K., K. B. Thapa, C. Cimpan, and H. Wenzel, 2018. Electronic waste and informal recycling in Kathmandu, Nepal: challenges and opportunities. *Journal of Material Cycles and Waste Management*, 20(1), 656–666. DOI: 10.1007/s10163-017-0610-8

Pariatamby, A., and D. Victor, 2013. *Policy trends of e-waste management in Asia*. 411–419. DOI: 10.1007/s10163-013-0136-7

Perkins, D. N., M. N. Brune Drisse, T. Nxele, and P. D. Sly, 2014. E-waste: a global hazard. *Annals of Global Health*, 80(4), 286–295. DOI: 10.1016/j.aogh.2014.10.001 PMID:25459330

Petrlik, J., J. Pucket, L. Bell, and J. DiGangi, 2019. *Weak controls: European e-waste poisons Africa's food chain*. *April*.

Rathore, P., S. Kota, and A. Chakrabarti, 2011. Sustainability through remanufacturing in India: a case study on mobile handsets. *Journal of Cleaner Production*, **19**(15), 1709–1722. DOI: 10.1016/j.jclepro.2011.06.016

Rawat, S., L. Verma, and J. Singh, 2020. Environmental hazards and management of e-waste. In *Environmental Concerns and Sustainable Development: Volume 2: Biodiversity, Soil and Waste Management* (pp. 381–398). Springer, Singapore.
DOI: 10.1007/978-981-13-6358-0_16
PMCID:PMC7278568

Sander, K., S. Schilling, T. Naoko, C. V. Rossem, J. Vernon, and C. George, 2007. *The Producer Responsibility Principle of the WEEE Directive*.

Satyal, U., 2017. Nepal lacks e-waste management lawsno title. *The Himalayan Post*.

Schluep, M., 2014. Informal waste recycling in developing countries. *Handbook of Recycling: State-of-the-Art for Practitioners, Analysts, and Scientists*, 439–444.
DOI: 10.1016/B978-0-12-396459-5.00029-5

Suja, F., R. A. Rahman, A. Yusof, and M. S. Masdar, 2014. *E-Waste Management Scenarios in Malaysia*. 2014.
DOI: 10.1155/2014/609169

Tong, X., and L. Yan, 2013. From legal transplants to sustainable transition: extended producer responsibility in Chinese waste electrical and electronic equipment management tong and yan epr in Chinese WEEE management. *Journal of Industrial Ecology*, 17(2), 199–212.
DOI: 10.1111/jiec.12013

Turaga, R. M. R., K. Bhaskar, S. Sinha, D. Hinchliffe, M. Hemkhaus, R. Arora, S. Chatterjee, D. S. Khetriwal, V. Radulovic, P. Singhal, and H. Sharma, 2019. E-waste management in India: issues and strategies:
DOI: 10.1177/0256090919880655

UNEP., 2015. *Illegally Traded and Dumped E-Waste Worth Up To $19 Billion Annually Poses Risks to Health, Deprives Countries of Resources, Says UNEP.* Environment Under Review.

Walther, G., J. Steinborn, T. S. Spengler, T. Luger, and C. Herrmann, 2010. Implementation of the WEEE-directive-economic effects and improvement potentials for reuse and recycling in Germany. *International Journal of Advanced Manufacturing Technology*, 47(5–8), 461–474.
DOI: 10.1007/s00170-009-2243-0

Wang, H., Y. Gu, L. Li, T. Liu, Y. Wu, and T. Zuo, 2017. Operating models and development trends in the extended producer responsibility system for waste electrical and electronic equipment. *Resources, Conservation and Recycling*, 127(May), 159–167.
DOI: 10.1016/j.resconrec.2017.09.002

Wang, L. K., M.-H. S. Wang, and Y. T. Hung, 2021. *Solid Waste Engineering and Management* (L. K. Wang, M.-H. S. Wang, & Y.-T. Hung (eds.); Vol. 1). Springer International Publishing.
DOI: 10.1007/978-3-030-84180-5

Yu, D. E. C., K. D. S. Yu, and R. R. Tan, 2020. Implications of the pandemic-induced electronic equipment demand surge on essential technology metals. *Cleaner and Responsible Consumption*, 1(November), 100005.
DOI: 10.1016/j.clrc.2020.100005

Zhang, K. S., 2020. *Turning Trash Into Treasure-A Comparative Study of E-Waste Recycling in China and the U.S. Through Systems Thinking*.

Hepatoprotective Potential of Selected Medicinal Plants: Evaluating their Antioxidant and Antibacterial Activities

Khem Raj Joshi[1*] and Sabina Parajuli[1]

[1]School of Health and Allied Sciences, Faculty of Health Sciences, Pokhara University, Nepal.

***CORRESPONDING AUTHOR:**

Khem Raj Joshi

Email:khemraj_pu@yahoo.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI :

https://doi.org/10.3126/njst.v21i2.67162

Date of Submission: Apr 27, 2023

Date of Acceptance: Apr 27, 2024

ABSTRACT

From the survey, a total of 41 plant species belonging to 30 families were found to be useful in the treatment of hepatic disorders. The mostly used were the whole plant of Cuscuta reflexa (85%) and fruits of *Carica papaya* (50%), *Saccharum officinarum* (46.5%) and *Cucurbita pepo* (44.5%). Herbal remedies were mostly prepared from freshly collected plants and used alone or with water. Among the documented species, six plant parts, that were least studied previously, were selected for the study of antioxidant and antibacterial activities. The extracts of *Diplazium stoliczkae,* rhizomes and leaves both, with IC_{50} value, 5.54 and 5.49 µg/mL respectively, close to that of standard, ascorbic acid (4.80 µg/mL) showed potent antioxidant activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. Also, the extracts of rhizomes and leaves of *D. stoliczkae* and bark of *Bombax Ceiba* showed antibacterial activity comparable to that of standard drugs at a concentration of 1 mg/mL.

Keywords: Hepatoprotective, Antioxidant, Antibacterial activity, *Diplazium stoliczkae, Bombax ceiba, Cuscuta reflexa*

1. Introduction

The use of wild plants or plant parts to fulfill the needs such as food, medicine and agricultural tools was base for indigenous people living in particular areas, so they have considerable knowledge on the uses of medicinal plant. Various ethnobotanical surveys on medicinal plants have been performed in various parts of Nepal (Bhattarai *et al*. 2006; Burlakoti & Kunwar 2008; Joshi & Joshi 2000; Kunwar *et al*. 2006; Manandhar 2002). However, there is a sparseness of documentation and a limited repository of knowledge regarding the utilization of wild medicinal plants in the country, particularly in the Kaski district, with a focus on specific diseases, such as liver disorders. The present research attempted to document the medicinal plants used by the people in the villages and the surrounding areas of the Kaski district.

Liver is a major organ attacked by reactive oxidative species or free radicals (Sanchez-Valle 2012). Oxidative stress is one of the pathological mechanisms that results in initiation and progression of various liver diseases, steatosis, fibrosis, cirrhosis, chronic hepatitis and hepatocellular carcinoma (Cichoz-Lach & Michalak 2014; Sha *et al*. 2015). So, various anti-oxidative therapy and antioxidants are

proposed to prevent and treat liver diseases. The clinical effects of antioxidants as adjuvants including vitamin E/C, mitoquinone, N-acetylcysteine, polaprezinc silymarin, silibinin and some antioxidant cocktail on chronic hepatitis C patients have been examined has shown clear benefit of antioxidants to interferon based therapy of hepatitis C virus (HCV) (Esrefoglu 2012). Due to presence of hydroxyl and carboxyl group, phenols and flavonoids can bind to the free radicals and deactivate them along with the ability to donate electron or the hydrogen atom to the unpaired free radicals. By donating the electron, phenolic compounds do not become the reactive species but possess the ability to donate to other free radicals (Michalak 2006).

Bacterial infections are very common in patients with cirrhosis and a major challenge for physicians caring for patients with liver diseases. Despite the recent improvements in the knowledge of pathogenesis, prevention, and management, bacterial infections still represent a major cause of morbidity and mortality among patients with cirrhosis (Jalan *et al*. 2014). Plants are the sources of various phytochemicals such as vitamins (A, C, E and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals that have antimicrobial and antioxidant activities (Madhuri & Pandey 2009). Various ethnobotanical surveys on medicinal plants have been performed in various parts of Nepal and several papers were published on different aspects of ethnobotany by different researchers. However, there is lack of documentation and store of knowledge on the uses of wild medicinal plants of the country, specifically, of Kaski district, based on specific diseases, such as liver disorders. The present research attempts to document the medicinal plants used by the people in the villages and the surrounding areas of the Kaski district.

2. Materials and Methods

2.1 Study area

Field study was carried out in the villages of Naudanda, Armala, Furse khola, Kotre, Ghandruk and in the surrounding areas of the Kaski district of Western Region of Nepal from September to December, 2015. The selection was random and the study design was cross-sectional. Key informants were identified after preliminary discussion with the people. Information on uses of the plants was collected by interviewing key informants of total 200 respondents of five different villages using a semi-structured questionnaire. The plants were identified in the field with the help of informants and their nomenclatures were found out with the help of literature. Those who have no idea about the medicinal plants were excluded.

Six selected plant extracts were taken for antioxidant and antibacterial activity. The plants selected were *Diplazium stoliczkae* (rhizomes), *D. stoliczkae* (leaves), *Cuscuta reflexa* (whole plant), *Bombax ceiba* (bark), *Ocimum basilicum* (seeds) and *Cirsium verutum* (roots). Those plants were identified and collected from survey area with the help of local people. The herbarium and voucher specimen (*Diplazium stoliczkae* (rhizomes) 13-2015, *D. stoliczkae* (leaves) 16-2025, *Cuscuta reflexa* (whole plant) 17-2015, *Bombax ceiba* (bark) 24-2015, *Ocimum basilicum* (seeds) 25-2015 and *Cirsium verutum* (roots) 28-2015). of those plants were stored Department of Pharmaceutical Sciences, School of Health and Allied Sciences, Pokhara University.

2.2 Extract Preparation

The collected plants were finely powdered and extracted using 70% methanol. Each plant sample of 50 g was mixed with 400 mL of 70% methanol by maceration for 4 days (96 hours). The 70% methanolic extracts were then filtered and the filtrates were dried using rotatory vacuum evaporator.

2.3 Antioxidant activity determination2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH radical free assay was measured according to the method of Kim *et al*. 2007 with some modifications (Kim *et al*. 2007). In brief, 2 mL of different extract solution (0.1 µg/mL, 1 µg/mL, 10 µg/mL and 100 µg/mL) of each plant sample were mixed with 2 mL of 60 µM DPPH solution. The mixture was allowed to stand for 30 minutes. The absorbance was measured at 517 nm using UV spectrophotometer. Radical scavenging activity of each sample was calculated by using following formula:

$$\text{Radical scavenging activity (\%)} = [(A_0 - A_S)/ A_0] \times 100$$

Where, A_0 = absorbance of control and A_S= absorbance of sample. Control is the test solution without sample. Similar process was done with ascorbic acid solution of concentrations (0.1 µg/mL, 1 µg/mL, 10 µg/mL and 100 µg/mL). Ascorbic acid was taken as standard solution.

2.4 Antibacterial activity determination

Pathogenic strains of *Staphylococcus aureus*, *Proteus vulgaris*, and *Escherichia coli* with their antibiotic resistance profiles were obtained from the National Public Health Laboratory (NPHL), Kathmandu. The Well diffusion method assay was according to Sen *et al*. 2012 with slight modifications (Sen & Batra 2012). An inoculum suspension was swabbed uniformly to solidified 20 mL Mueller-Hinton agar (MHA) for bacteria, and the inoculum was allowed to dry for 5 minutes. Holes of 6 mm in diameter were made in the seeded agar using Glass Pasteur pipettes. Aliquot of 50 µL from each plant crude extract (50 mg/mL) was added into each well on the seeded medium and allowed to stand on the bench for 2 hours for proper diffusion and thereafter incubated at 37 °C for 24 hours. Ofloxacin and cefpodoxime (1 mg/ml) were used as standards. 10% DMSO was used as positive control. The resulting inhibition zones were measured in millimeters (mm). Larger the zone of inhibition, higher is the antibacterial activity.

2.5 Determination of total phenol

Total phenols were determined by Folin Ciocalteu reagent with some modifications (Pourmorad *et al*. 2006). In brief, 200 µL of each extract solution (1 mg/mL) was mixed with 1800 µL of distilled water and 2 mL of Folin reagent. After standing for 3 minutes, 2 ml of 10% sodium carbonate was mixed and shaken. The mixture was allowed to stand for 1 hour and the absorbance was measured at 760 nm. Gallic acid was taken as a standard phenolic compound. Different concentrations of gallic acid (500 mg/L, 400 mg/L, 300 mg/L, 200 mg/L, 100 mg/L, and 50 mg/L) were prepared. Total phenol values were expressed as mg gallic acid equivalent per gram dry extract weight.

2.6 Determination of flavonoids content

Aluminum chloride colorimetric method was used for flavonoid determination (with some modifications (Chang *et al*. 2002). In brief, 1 mL of each plant extract solution (1 mg/mL) was mixed with 4 mL of distilled water. Then, 300 µL of 5% sodium nitrite was added. After 5 minutes, 300 µL 20% aluminum chloride was added and allowed to stand for 6 minutes. Then after, 2 mL of 1M sodium hydroxide was added. The mixture was shaken and the absorbance was measured at 510 nm using UV spectrophotometer. Quercetin was taken as standard flavonoid compound. Different concentrations of quercetin (500 mg/L, 400 mg/L, 300 mg/L, 200 mg/L, 100 mg/L, and 50 mg/L) were prepared. Total flavonoid values were expressed as mg quercetin equivalent per gram dry extract weight.

3. Results

From the survey, a total of 41 plant species belonging to 30 families were found to be useful in the treatment of jaundice and hepatitis. The mostly used plants for hepatic ailments are shown in Fig. 1. Eighty five percent of the total respondents have knowledge about the whole plant of *Cuscuta reflexa.* The modes of uses were documented and herbal remedies were mostly prepared from freshly collected plants used alone or with water. The preferred method of preparation was crushing while doses of most plants were as per required till cure. The knowledge about the medicinal plants were mostly found to be transformed from ancestors and other by local practitioners, learnt from secondary sources such as studying books, literatures, friends, relatives, etc.

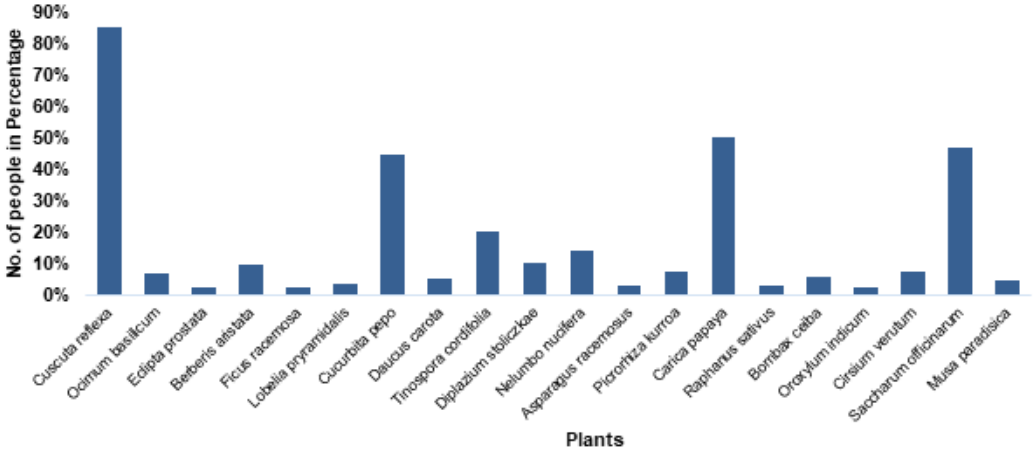


Fig. 1: Graph representing the widely used plants in hepatic disorders by local people

According to the survey conducted in this study, in addition to *Cuscuta reflexa, Cucurbita pepo, Carica papaya,* and *Sacaharum officinarum* were seen as highly preferred plants for treating hepatic disorders by local people. Among the documented species, six plant parts, that were least studied previously, were selected for the study of antioxidant and antibacterial activities.

3.1 Antioxidant activity determination

The hydrogen atom or electron donation ability of each plant extract against DPPH free radical was measured from the bleaching of violet colored methanol solution of DPPH. The DPPH radical absorbs at 517 nm and antioxidant activity was determined by monitoring the decrease in absorbance. DPPH radical scavenging activity of each sample at different concentrations is shown in Fig. 2. Results were reported as IC_{50} which is defined as the amount of antioxidant required to inhibit 50% of DPPH free radicals under the experimental conditions. Among the studied plant extracts, the extract of *D. stoliczkae* (rhizomes), *D. stoliczkae* (leaves) and *B. ceiba* (bark) showed IC_{50} value close to that of standard, ascorbic acid (Fig. 3).

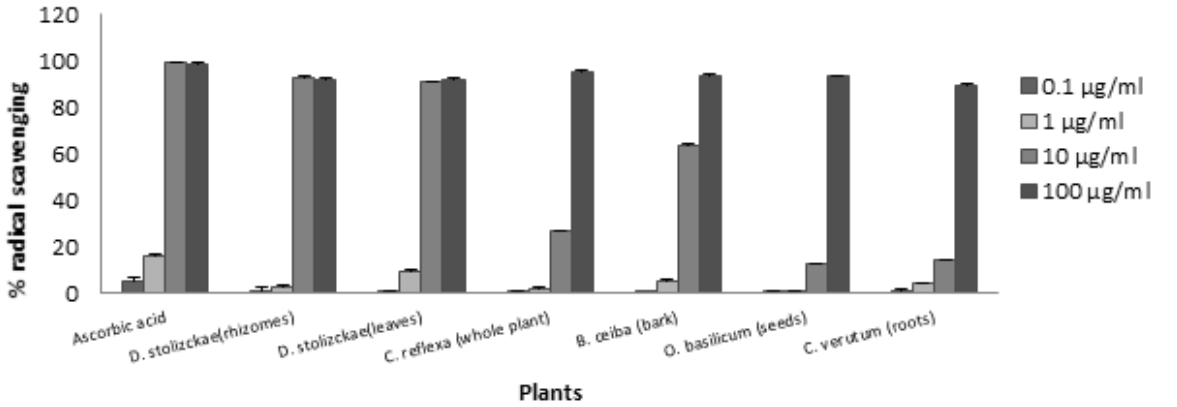


Fig. 2: Graphical representation of percentage scavenging of DPPH free radicals by extracts/ ascorbic acid at 517 nm.

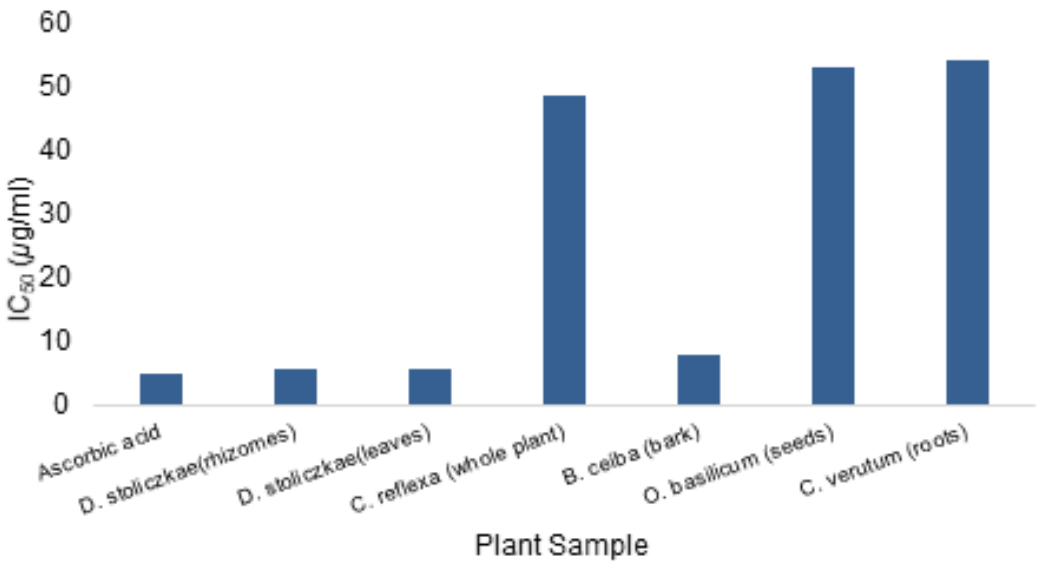


Fig. 3: Graphical representation of IC_{50} (µg/mL) values of plant extracts for free radicals

3.2 Antibacterial activity determination

Each plant crude extracts (50 mg/mL) were measured and compared with the zone of inhibitions produced by the (1 mg/mL) standard antibiotics ofloxacin and cefpodoxime. Among the six sample extracts the rhizomes and leaves of *D. stoliczkae* and bark of *B. Ceiba* showed better antibacterial activity against all test organisms while other extracts showed no zone of inhibition. The inhibition zone diameter is given in the table

Table 1: Antibacterial activity

SN	Plant sample/Conc.	Diameter of inhibition zone (mm) *		
		Gram -ve bacteria		Gram +ve bacteria
		E. coli	*P. vulgaris*	*S. aureus*
		50 mg/mL	50 mg/mL	50mg/mL
1	*D. stoliczkae* (rhizomes)	27	32	10
2	*D. stoliczkae* (leaves)	29	33	10
3	*B. ceiba* (bark)	30	30	11
4	Ofloxacin (1mg/mL)	42	47	38
5	Cefpodoxime (1mg/mL)	33	32	18
6	10% DMSO (1mg/mL)	0	0	0

*Diameter of inhibition zones includes the diameter of well (6 mm)

The standard drugs ofloxacin and cefpodoxime showed higher zone of inhibition as compared to sample extracts.

3.3 Determination of total phenol

The extract that displayed the higher concentration of total phenols were that of *D. stoliczkae* (rhizomes), *D. stoliczkae* (leaves) and *B. ceiba* (bark) i.e. 380 ± 3.81, 375.69 ± 0.49 and 254.71 ± 2.43 mg GAE/g dry extract wt. respectively while other extracts showed lower phenol content.

3.4 Determination of flavonoids content

The extract that displayed the higher concentration of flavonoids were that of *D. stoliczkae* (rhizomes), *D. stoliczkae* (leaves) and *B. ceiba* (bark) i.e. 401.1 ± 2.80, 372.1 ± 5.70 and 307.6 ± 2.10 mg QE/g dry extract wt. respectively while other extracts showed lower flavonoids content.

4. Discussion

The present study was performed to identify the hepatoprotective plants from Western region of Nepal, around Kaski district and evaluate their biological importance scientifically. For this, ethnomedicinal survey was carried out on plants that have been used by local people for hepatic ailments such as jaundice and hepatitis. On basis of the survey, some plants were selected for their biological activities that have been less studied scientifically. Most commonly used was found to be *C. reflexa,* about 85% of the respondents were aware about the plant. People also mostly used the fruits such as *C. papaya*, *S. officinarum* and *C. pepo* and found useful for efficient cure of liver diseases. The survey was useful as it also revealed those medicinal plants that were widely available around our locality but were less studied scientifically for liver ailments such as *D. stoliczkae, C. verutum, O. basilicum, B. Ceiba*, etc. Herbal remedies for liver disorders have been widely used and has been popularized world over by leading pharmaceuticals and the most widely used medicines for liver ailments are based on herbal remedies (Singh *et al*. 2012).

From the data available from the survey, six plants were selected for determining their antioxidant and antimicrobial activities. Antioxidant activity was determined by well-established method, DPPH assay and further evaluation of total phenol and flavonoid content. DPPH forms a stable molecule on accepting an electron or a hydrogen atom and thus has applications in determination of radical scavenging activity of natural products as well as synthetic compounds (Bhuiyan et al 2019). Plant phenols act as primary antioxidants or free radical terminators while flavonoid as one of the most important natural phenols, possesses radical scavenging

properties. The presence of a certain structure and particularly hydroxyl position in the molecule determine their antioxidant properties; in general, these properties depend on the ability to donate hydrogen or electron to a free radical (Luis *et al*. 2009). Liver is a major organ attacked by reactive oxidative species or free radicals and presence of antioxidants can play role to scavenge such reactive species and protect liver from injuries and diseases (Sanchez-Valle *et al*. 2012). The plants containing polyphenols and flavonoids can be useful for their role in such degenerative diseases. Among the studied plant extracts, the rhizomes and leaves extracts of *D. stoliczkae* and bark extracts of *B. ceiba* showed radical scavenging activity and IC_{50} value close to that of standard. Also these plant extracts showed the higher phenol and flavonoid content than other plant extracts.

Among the studied plant extracts, the rhizomes and leaf extracts of *D. stoliczkae*, as well as the bark extracts of *B. ceiba*, exhibited excellent radical scavenging and antibacterial activities. This was attributed to these specific plant extracts showcasing higher phenol and flavonoid content compared to other plant extracts.

From this survey, a total of 41 plant species, including *D. stoliczkae* and *B. ceiba*, were identified as being beneficial in the treatment of hepatic disorders

These in vitro assays indicate that these plant extracts could be significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. In a research conducted by Gandhare and colleagues in 2010, an antioxidant assay was performed using the bark extract of *B. ceiba*. The findings of the study demonstrated the antioxidant capabilities of the plant. It was suggested that phenols and flavonoids present in the extract could be accountable for this activity. This conclusion was supported by a subsequent study conducted by Wanjari *et al*. in 2016. The present study also proved the antioxidant potential and higher phenol and flavonoid content of the bark of *B. ceiba*. Previous study of *D. stoliczkae* for its antioxidant activity by Parajuli *et al*. 2012, also revealed the better radical scavenging activity and higher flavonoid and phenol content of the rhizomes extract of the plant (Parajuli *et al*. 2012). The present study proved not only the rhizomes extract of the plant *D. stoliczkae*, but also the leaves also show better antioxidant activity. This can be useful for further biological activity study. The present study also determined the antibacterial activity of the selected plant extracts. Despite the widespread use of broad spectrum antibiotics, bacterial infection is responsible for up to a quarter of the deaths of patients with liver disease. The plant with antibacterial activities can be useful for protection of liver from bacterial infections (Wyke 1987; Semwal *et al*. 2021). The above study showed the better antibacterial effect of the extracts of *D. stoliczkae* and *B. ceiba* as well. The result, therefore, can be useful for further analysis of hepatoprotective activities of these plants. A study by Ravi *et al*., 2010, showed the hepatoprotective activity of *B. ceiba* Linn against drug-induced toxicity in experimental rats (Ravi *et al*. 2010). Currently, there hasn't been any experimental research conducted on the plant *D. stoliczkae.* However, based on the findings presented earlier and by comparing them with results from other plants, it's evident that the rhizomes and leaf extracts of *D. stoliczkae* exhibit superior in vitro activities. This suggests potential benefits for further investigation into its biological properties through in vivo studies, particularly to confirm its potential hepatoprotective effects.

5. Conclusion

The above study was useful to find out the medicinal plants that can be used for hepatoprotection from the survey and biological assays as well. The study concluded the better biological activity of the plant *D. stoliczkae.* The plant can be further taken for evaluation of hepatoprotective and other pharmacological activities evaluation that can be useful for newer drugs development in modern medicine for treatment of various ailments.

References

Acharya, K.M., and M. Acharya, 2010. Traditional Knowledge on Medicinal Plants Used for the Treatment of Livestock Diseases in Sardikhola VDC, Kaski, Nepal, *Journal of Medicinal Plants Research*, **4**(2), 235-239.

Bhattarai, S., R. P. Chaudhary, and R. S. Taylor, 2006. Ethnomedicinal Plants Used by the People of Manang District, Central Nepal, *Journal of Ethnobiology and Ethnomedicine*, **2**(4), 1-8. DOI: 10.1186/1746-4269-2-41 PMID:17020612 PMCID:PMC1618386

Burlakoti, C., and R. M. Kunwar, 2008. Folk Herbal Medicines of Mahakali Watershed Area, Nepal, *Medicinal Plants in Nepal: An Anthology of Contemporary Research*, pp. 187-193.

Cichoz-Lach, H., and A. Michalak, 2014. Oxidative Stress as a Crucial Factor in Liver Diseases, *World Journal of. Gastroenterology*, 20(25), 8082–8091. DOI: 10.3748/wjg.v20.i25.8082 PMID:25009380 PMCID:PMC4081679

Joshi, A. R., and K. Joshi, 2000. Indigenous Knowledge and Uses of Medicinal Plants by Local Communities of the Kali Gandaki Watershed Area, Nepal, *Journal of Ethnopharmacology*, 73(1-2), 175-183. DOI: 10.1016/S0378-8741(00)00301-9 PMID:11025154

Kunwar, R. M., B. K. Nepal, H. B. Kshettri, S. K. Rai, and R. W. Bussman, 2006. Ethnomedicine in Himalaya: A Case Study from Dolpa, Humla, Jumla and Mustang Districts of Nepal, *Journal of Ethnobiology and Ethnomedicine*, 2(27), 1-6. DOI: 10.1186/1746-4269-2-27 PMID:16749924 PMCID:PMC1513199

Manandhar, N. P., 2002. *Plants and People of Nepal*, Timber Press Inc., Portland, pp 75-441.

Sanchez-Valle, V., N. C. Chavez-Tapia, M. Uribe, and N. Mendez-Sanchez, 2012. Role of Oxidative Stress and Molecular Changes in Liver Fbrosis: A Review, *Current Medicinal Chemistry*, **19**, 4850–4860. DOI: 10.2174/092986712803341520 PMID: 22709007

Sha, L., T. Hor-Yue, W. Ning, Z. Zhang-Jin, L. Lixing, W. Chi-Woon, and F. Yibin, 2015. The Role of Oxidative Stress and Antioxidants in Liver Diseases. *International Journal of Molecular Sciences,* **16**, 26087–26124. DOI: 10.3390/ijms161125942 PMID:26540040 PMCID:PMC4661801

Esrefoglu, M., 2012. Oxidative Stress and Benefits of Antioxidant Agents in Acute and Chronic Hepatitis, *Hepatitis Monthly*, **12**, 160–167. DOI: 10.5812/hepatmon.837 PMID: 22550523 PMCID: PMC3339415

Michalak, A., 2006. Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal Stress, *Polish Journal of Environmental Studies*, **15**(4), 523-530.

Jalan, R., J. Fernandez, R. Wiest, B. Schnabl, R. Moreau, P. Angeli, V. Stadlbauer, T. Gustot, M. Bernardi, R. Canton, A. Albillos, F. Lammert, A. Wilmer, R. Mookerjee, J. Vila, R. Garcia-Martinez, J. Wendon, J. Such, J. Cordoba, A. Sanyal, G. Garcia-Tsao, V. Arroyo, A. Burroughs, and P. Gines, 2013. Bacterial infections in cirrhosis: A position statement based on the EASL Special Conference, *Journal of Hepatology*, 2014, 60, 1310–1324. DOI: 10.1016/j.jhep.2014.01.024 PMID:24530646

Madhuri, S., and G. Pandey, 2009. Some Anticancer Medicinal Plants of Foreign Origin, *Current Science*, 96(6), 25.

Kim, M. K., H. S. Lee, E. J. Kim, N. H. Won, Y. M. Chi, B. C. Kim, and K. W. Lee, 2007. Protective Effect of Aqueous Extract of *Perilla frutescens* on *tert*-butyl hydroperoxide-induced Oxidative Hepatotoxicity in Rats,*Food and Chemical Toxicology*, 45, 1738-1744. DOI: 10.1016/j.fct.2007.03.009 PMID:17467864

Sen, A., and A. Batra, 2012. Evaluation of Antimicrobial Activity of Different Solvent Extracts of Medicinal Plant: *Melia Azedarach* L**,** *International Journal of Current Pharmaceutical Research*, **4**(2), 67-73.

Pourmorad, F., S. J. Hosseinimehr, and N. Shahabimajd, 2006. Antioxidant Activity, Phenol and Flavonoid Contents of Some Selected Iranian Medicinal Plants, *African Journal of Biotechnology*, **5**(11), 1142-1145.

Chang, C.C., M. H. Yang, H. M. Wen, and J. C. Chern, 2002. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods, *Journal of Food and Drug Analysis*, **10**(3), 178-182. DOI: 10.38212/2224-6614.2748

Singh, A. G., A. Kumar, and D. D. Tewari, 2012. An Ethnobotanical Survey of Medicinal Plants Used in Terai Forest of Western Nepal, *Journal of Ethnobiology and Ethnomedicine*, **8**(9), 1-14. DOI: 10.1186/1746-4269-8-19 PMID: 22591592 PMCID: PMC3473258

Bhuiyan, M. A. R., M. Z. Hoque, and S. J. Hossain, 2009. Free Radical Scavenging Activities of *Zizyphus mauritiana*, *World Journal of Agriculture Sciences*, **5**(3), 318-322.

Luis, A., F. Domingues, C. Gil, and A.P. Duarte, 2009. Antioxidant Activity of Extracts of Portuguese Shrubs: *Pterospartum tridentatum*, *Cytisus scoparius* and *Erica* Spp, *Journal of Medicinal Plants Research*, **3**(11), 886-893.

Gandhare, B., N. Soni, and H. J. Dhongade, 2010. In Vitro Antioxidant Activity of *Bombax Ceiba*, *International Journal of Biomedical Research*, **1**(2), 31-36.
DOI: 10.7439/ijbr.v1i2.57

Parajuli, S., N. P. Tilija, S. Parajuli, and N. P. Jammarkattel, 2012. Antioxidant activity, Total Phenol and Flavonoid Contents in Selected Medicinal Plants of Nepal, *Journal of Health and Allied Sciences*, **2**(1), 27-31.
DOI: 10.37107/jhas.71

Wyke, R.J., 1987. Problems of Bacterial Infection in Patients with Liver Disease, *Gut*, **28**, 623-641.
DOI: 10.1136/gut.28.5.623
PMID:3297941 PMCID:PMC1432873

Ravi, V., S. S. Patel, N. K. Verma, D. Dutta, and T. S. M. Saleem, 2010. Hepatoprotective Activity of *Bombax ceiba* Linn against Isoniazid and Rifampicin-induced Toxicity in Experimental Rats, *International Journal of Applied Research in Natural Products*, **3**(3),19-26.

Wanjari, M.M., R. Gangoria, Y.N. Dey, S.N. Gaidhani, N.K. Pandey, and A.D. Jadhav, 2016. Hepatoprotective and antioxidant activity of *Bombax ceiba* flowers against carbon tetrachloride-induced hepatotoxicity in rats. *Hepatoma Research*, 2: 144-50.
DOI: 10.20517/2394-5079.2015.55

Semwal, P., S. Painuli, K. M. Painuli, G. Antika, T. B. Tumer, A. Thapliyal, W.N. Setzer, M. Martorell, M. M. Alshehri, Y. Taheri, S. D. Daştan, S. A. Ayatollahi, A. T. Petkoska, J. Sharifi-Rad, and W. C. Cho, 2021. *Diplazium esculentum* (Retz.) Sw.: Ethnomedicinal, Phytochemical, and Pharmacological Overview of the Himalayan Ferns. Oxid Med Cell Longev. Sep 2;2021:1917890.
DOI: 10.1155/2021/1917890
PMID: 34512863 PMCID: PMC8433033

Effects of In-situ Clay-induced Formation Damage on Oil Recovery During Low-Salinity-Based Enhanced Oil Recovery Method in a Sandstone Reservoir of Upper Assam Basin, India

Nayan Medhi[1*]

[1]*Department of Petroleum Engineering, Dibrugarh University, Dibrugarh-786004*

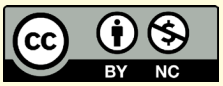
***CORRESPONDING AUTHOR:**
Nayan Medhi
Email:nmedhi.duiet@dibru.ac.in

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI :
https://doi.org/10.3126/njst.v21i2.67163

Date of Submission: Dec 13, 2022

Date of Acceptance: Apr 30, 2024



ABSTRACT

Clay-Induced formation damage is a worldwide problem in the petroleum industry, which is caused by the swelling and migration of clays and subsequent plugging of the pore throats. The In-Situ formation damage by clay minerals in sandstone reservoirs is governed by the physicochemical factors that control the stability and transport of the clays. The study presented here focuses on the effects of In-Situ Clay-Induced Formation Damage on oil recovery during Low Salinity Waterflooding (LSW) in the Tipam Reservoir Sandstone of Upper Assam Basin, India. Analysis of reservoir rock, formation brine, and crude oil shows the feasibility of LSW in the study area. The paper describes the alteration of rock permeability and porosity in a series of core flooding experiments using low-salinity brine. It is observed that the permeability and porosity of the flooded core plugs decrease during LSW. The SEM analysis of the fines migrated along with the effluent water during core flooding shows the presence of Kaolinite, Illite, and Mixed-layer. The study shows that the permeability reduction occurs during LSW through the plugging of pore throats which may be due to some mechanical and chemical processes like migration and swelling of clays. This plugging can increase the oil recovery by enhancing the Sweep Efficiency. Also, the migrated clay minerals can enhance the oil recovery by wettability modification and reduction of oil-water Interfacial Tension (IFT). Further, the permeability decline in the Swept Zone may improve the LSW performance by increasing the water breakthrough time and reducing the water cut.

Keywords: Basin, Core Flooding, LSW, Permeability, Wettability

1. Introduction

Formation Damage can be defined as a reduction in the initial permeability of the reservoir rock near the vicinity of the wellbore following various operations such as drilling, completion, stimulation, production, and workover operations (Wilson *et al*. 2014). It includes flow restrictions due to the reduction in permeability in the near-wellbore regions, alteration of the relative permeability to the hydrocarbon phase, and unintended flow restrictions in the completion itself. One of the main reasons behind the productivity reduction in a hydrocarbon reservoir and injectivity problem in a water flood project is the formation damage. Sometimes, the information available makes it very difficult to quantify formation damage that can happen anywhere and anytime (Radwan *et al*. 2019; Civan 2015; Portier *et al*. 2007; Bennion *et al*. 1995). Therefore, it is better to avoid formation damage than to restore it because restoring formation damage may result in additional damage to the formation (Al-Hetari 2017).

Formation damage can be caused by different Mechanical, Chemical, Thermal, and Biological methods (Al-Hetari 2017; Ezenweichu & Laditan 2015). The Mechanical formation damage results from the migration of clays (fines migration) like Kaolinite and Illite and the subsequent blocking of some of the pore throats in the reservoir rock (Mohan *et al*. 1993). The three basic steps of formation damage by fines migration are the presence of fines in the rock, migration of fines, and trapping of the fines in place (Xiao *et al*. 2017). This fines migration in a petroleum reservoir depends on different factors like mineralogical composition, porosity & permeability of the rock, injection & formation brine salinity, brine pH, temperature, Residual Oil Saturation (ROS), the fractional flow of oil & water, rock wettability, oil polarity and drag force (He *et al*. 2012; He *et al*. 2013; Hibbeler *et al*. 2003; Huang *et al*. 2008; Kalfayan & Watkins 1990). The mineralogical composition of a reservoir rock plays a major role in fines migration as the clay minerals in the rock migrate along with the flowing fluid under favorable conditions. It is observed that low salinity and high pH of brine cause fines to be released from the pore walls (Vaidya & Fogler 1990; Tang & Morrow 1999). It is also found that the permeability of a rock sample decreases with increasing temperature due to fine migration (Rosenbrand *et al*. 2015). Apart from the fines migration, blocking of pore throats by external solids, perforation damage, and phase trapping are some other mechanical methods of formation damage.

The Chemical Methods of formation damage include mainly swelling of clays and precipitation of insoluble solids (Al-Hetari 2017). Swelling of clay occurs when the initial equilibrium state of the Crude Oil/Brine/Rock (COBR) system is disturbed, especially during the injection of low-salinity brine into a reservoir containing clay. Clay swelling also occurs during drilling, completion, stimulation, and work over operation. Clay minerals are built from layers (sheets) of SiO_4 tetrahedrons and octahedrons like $Al_2(OH)_6$)n or ((Fe or $Mg)_3(OH)_6$)n. These tetrahedral and octahedral layers are stacked on top of each other.The swelling of the clays is due to the increase in interlayer spacing in clay particles (Zhou *et al*. 1996). These clay minerals have Cation Exchange Capacity (CEC), which is a measure of their ability to attract and hold cations from the bulkfluid (Hamilton 2009). Earlier studies have found that Kaolinite, Illite, and Smectite clays have high CEC that can cause a local pH increase in the vicinity of the clay surfaces (Austad *et al*. 2010; Hughes *et al*. 2010). This pH increase in the surrounding solution increases the detachment of fines from the pore walls, as mentioned above. On the other hand, the precipitation of insoluble solids in the porespaces is caused due to chemical reactions or disturbances in thermodynamic equilibrium. When the chemistry of the injection brine and reservoir fluid is different, there is always a possibility of formation damage through clay swelling and insoluble solid precipitation. These precipitants can be either from the brine (inorganic compounds) or from the crude oil (organic species), where the precipitation occurs due to the alteration of temperature or pressure near the vicinity of the wellbore or from the changes inthe injected fluid composition (Economides *et al*. 1994).

Formation damage by Thermal Methods occurs during some high-temperature Enhanced Oil Recovery (EOR) operations like In-situ Combustion and Steam Injection. These high-temperature operations may lead to mineral dissolution and transformation, where rock minerals are catalyzed and transformed from the earlier nonreactive clays to reactive products, which result in clay swelling, clay deflocculation, and reduction of rock permeability (Faergestad 2016). On the other hand, Biological Formation Damage is the result of the chemical interaction between the bacteria and food

substances present in sandstone reservoirs. It is widely acknowledged that bacteria are present in sandstone reservoirs (Scott & Davies 1993). The introduction of bacteria into the formation can also occur during drilling, water flooding, Microbial Enhanced Oil Recovery (MEOR), and some other operations. These bacteria can result in partial plugging of the pore by bacterial slimes and precipitation of insoluble precipitants (Shibulal *et al*. 2014).

It has been recognized that clay minerals can cause significant damage to the formation of a hydrocarbon reservoir which can reduce the permeability of a reservoir by more than 90% (Zhou *et al*. 1995). Earlier studies have found that Low Salinity Water flooding (LSW), which is a new Enhanced Oil Recovery method, causes In-Situ formation damage (Tang and Morrow 1999). In LSW, injection water salinity is comparatively lower than the reservoir brine salinity, which causes the Smectite and Mixed-layer to swell along with the breakage of fines that are in contact. These fines are then migrated (Swelling Induced Migration) along with the clay minerals, which are directly migrated (mainly Kaolinite and Illite) by the 'Fine Migration' mechanism. These migrated, and swelling clays can block some of the pore throats in the reservoir rock resulting in a reduction of permeability. Thus, LSW can cause In-Situ Clay-Induced formation damage in the swept area through Clay Swelling, Clay Migration, and Swelling Induced Migration (Tang & Morrow 1999; Mohan *et al*. 1993). Therefore, proper design of the Low-Salinity-Based EOR method is very important to reduce the formation damage, which includes fines management (Civan 2007, 2010; Fogden 2012). However, although the Clay-Induced formation damage from Low-Salinity-Based EOR reduces the connectivity in the formation, it can also enhance the oil production through the improvement of Sweep Efficiency, increasing the water breakthrough time and reducing the water-cut (RezaeiDoust 2009; Zeinijahromi *et al*. 2011). In addition to this, the migrated clay minerals can reduce the Residual Oil Saturation through the wettability alteration of rock and reduction of oil-brine Interfacial Tension (Tang & Morrow 1999; Bruin 2012). In this paper, a study has been made on the effects of In-Situ Clay-Induced formation damage on oil recovery during LSW in the Tipam Reservoir Sandstone of Upper Assam Basin.

2. Materials and Methods

For the present study, the Reservoir Rock sample, Crude Oil sample, and some Formation Brine data were collected from the study area. The Reservoir Rock samples were collected from five different wells from the area under study, whereas the Crude Oil sample was collected directly from a producing well located in the study area. All the rock samples were from the Tipam Reservoir Sandstone of the Upper Assam Basin. The depth range of the rock samples are 2893.00 m-2902.00 m, 2962.00 m-2970.00 m, 2898.43 m-2906.00 m, 2964.00 m- 2973.00 m, and 2853.00 m -2861.00 m.

2.1 Analysis of Reservoir Fluids

Crude Oil analysis was done to determine asphaltene content, resin content, Water Cut, density, Pour Point, wax content, and Acid Number as per the IP143, IOC-AOD, IP358, IP160, IP015, UOP 46-85, and IP001 standards, respectively. The analysis shows that Crude Oil contains resin and asphaltene with an Acid Number of 0.58 (Medhi 2018). Earlier studies show that the polar compounds (resin and asphaltene) and high Acid Number (>0.2) of oil are suitable for the application of LSW in an oil reservoir (Lager *et al*. 2008a; Ehrlich *et al*. 1974; Ehrlich & Wygal 1977).

The Formation Brine analysis shows that its salinity is 1404 ppm (as NaCl) which contains Ca^{2+} (6 ppm) and Mg^{2+} (8 ppm) (Medhi & Das 2015). According to Lager *et al*. (2008a) and Lager *et al*. (2006-8), the presence of Ca^{2+} and Mg^{2+} in the formation brine is the mandatory ions required for obtaining the LowSal Effect (LSE).

2.2 Petrographic Analysis

Petrographic analysis of rock helps to determine the mineralogy and texture of the rock types. The study also provides information about the diagenetic history, cementation, degree of compaction, effect of pressure solution, and subsidence of the basin of deposition (Das 1996). In the present work, mainly the mineralogy of the reservoir rocks is studied along with the texture of the rocks to some extent to evaluate their role in formation damage and oil recovery during LSW. In this study, the minerals in the rock samples were identified with the help of Thin Section Analysis (Fig. 1), X-Ray Diffraction (XRD) Analysis (Fig. 2 & 3), and Scanning Electron Microscopic (SEM) study (Fig. 4).

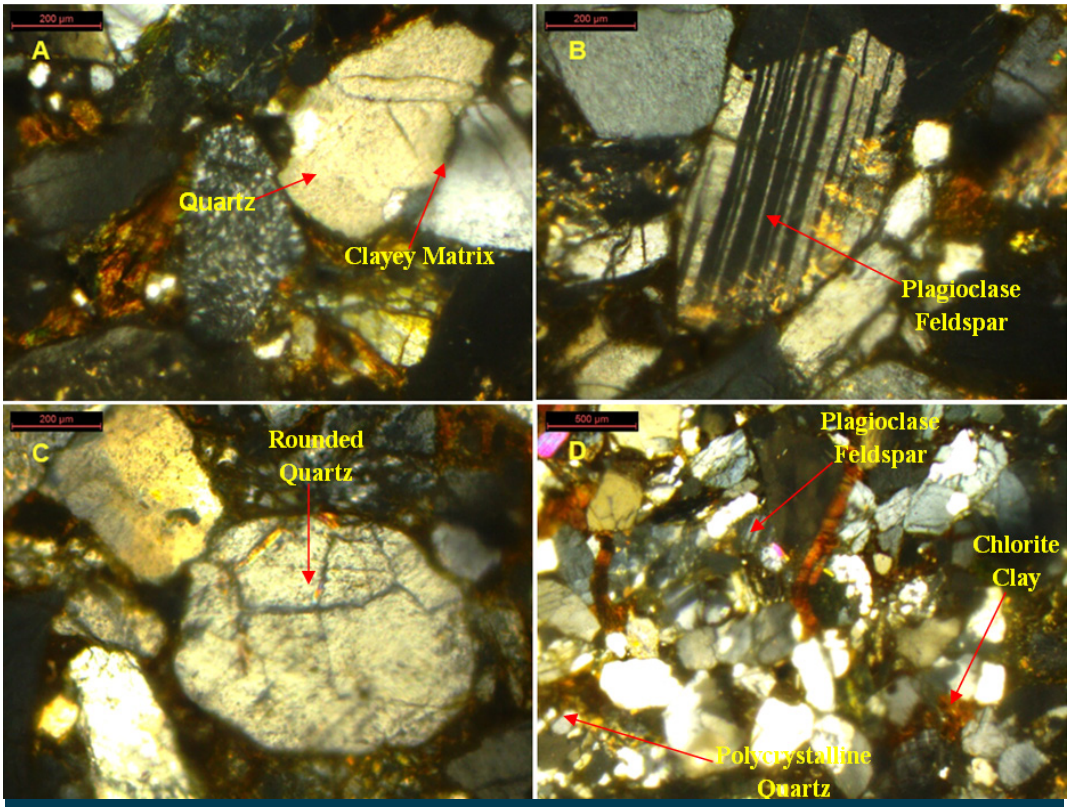


Fig. 1: Photomicrographs of the rock samples of the study area showing Quartz, Clayey Matrix (A); Plagioclase Feldspar (B); Rounded Quartz (C); Plagioclase Feldspar, Polycrystalline Quartz, Chlorite Clay (D).

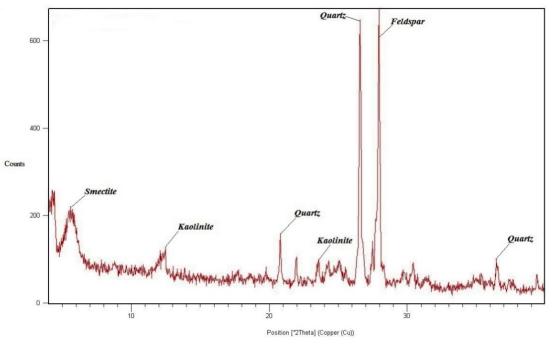


Fig. 2: X-Ray Diffractogram of the'Rock Sample A' showing Kaolinite, Smectite, Feldspar, and Quartz (Medhi 2016)

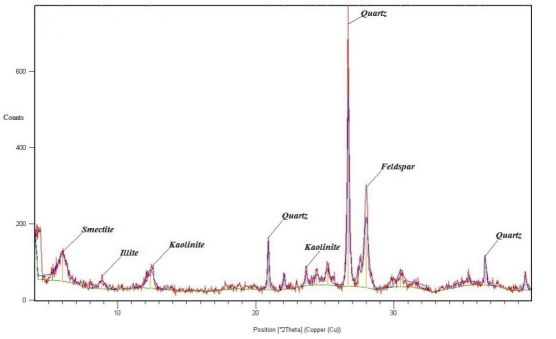


Fig. 3: X-Ray Diffractogram of the 'Rock Sample B'showing Kaolinite, Illite, Smectite, Feldspar, and Quartz (Medhi 2016)

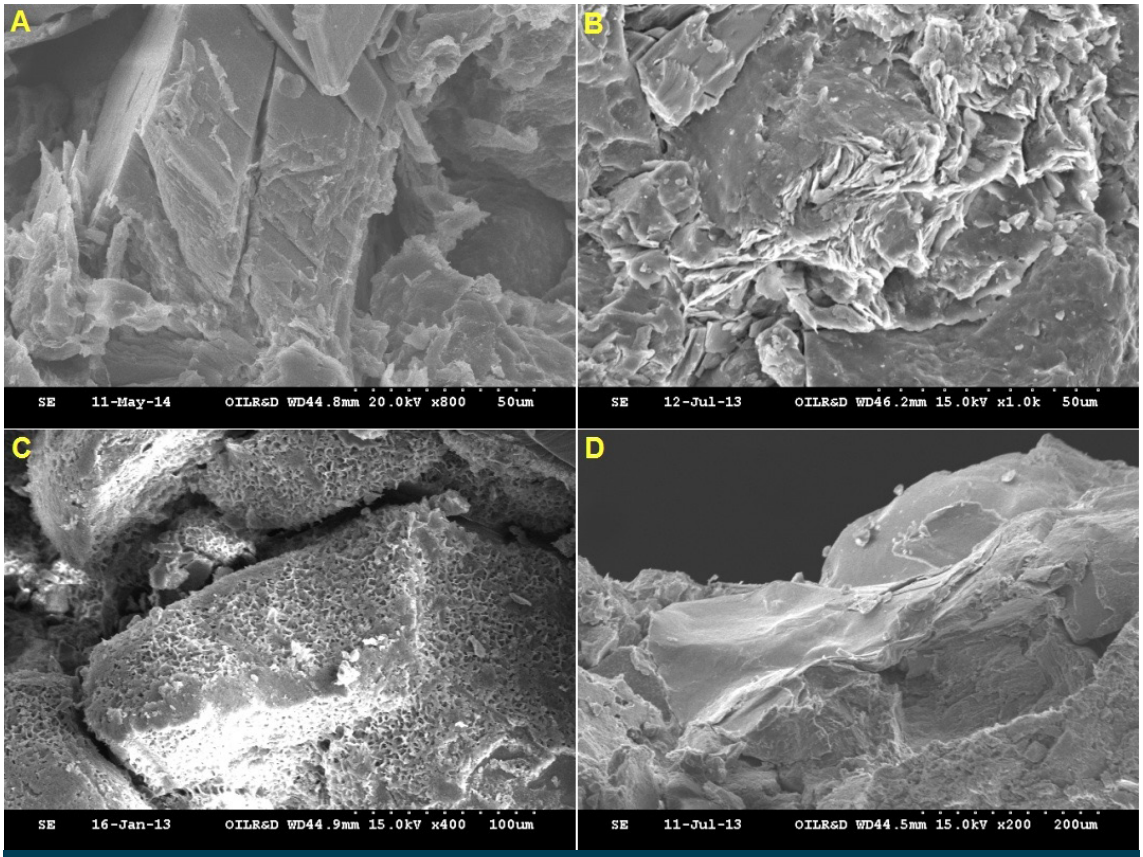


Fig. 4: SEM Photomicrograph of the rock samples of the study area showing Feldspar (A), Illite (B), Smectite (C), and Mica (D).

2.3 Preparation of Core Plugs

For this study, eight Sandstone Core Plugs (1.5-inch dia.) were plugged from the Conventional Core Sample using the Core Plugging Machine, which are then end-faced. The Core Plugs were then cleaned using Soxhlet Apparatus (Fig. 5) and Ultrasonic Cleaner. The cleaned Core Plugs were dried properly in the Humidity Cabinet to remove the liquid present inside the plugs. As the reservoir rock contains a high amount of clay (as per API RP 40. 1998), as observed in the petrographic analysis, the drying process was done at a Dry Bulb Temperature of 63 ^{0}C and Relative Humidity of 40% (Table 1). The clean and dry Core Plugs were then preserved properly for further laboratory experiments. Some of the prepared Core Plugs are shown in Fig. 6

Fig. 5: Core Plugs Cleaning by Soxhlet Apparatus

Table 1: Core Plug Drying Methods and Required Temperature (API RP 40. 1998)

Sl. No.	Rock Type	Method	Temperature (0C)
1	Sandstone (Low clay content)	i) Conventional Oven ii) Vacuum Oven	116 90
2	Sandstone (High clay content)	Humidity oven, 40% relative humidity	63
3	Carbonate	i) Conventional oven ii) Vacuum oven	116 90
4	Gypsum-bearing	Humidity oven, 40% relative humidity	60
5	Shale or other high clay rock	Humidity oven, 40% relative humidity Conventional vacuum	60

Fig. 6: Clean and Dry Core Plugs

2.4 Determination of Porosity and Permeability

After measuring the Effective Porosity of the clean and dry Core Plugs using Coretest TPI-219 Instructional Helium Porosimeter, the Air Permeabilities of the Core Plugs were determined using NDP-605 NanoDarcy Permeameter (Fig. 7), and corresponding Theoretical Liquid Permeabilities were determined based on Klinkenberg Effect. The plot of Air Permeability (md) and Inverse of Mean Pressure (1/Pm) for the Core Plug-3 are shown in Fig. 8. The porosity and the permeability of the eight Core Plugs are given in Table 2.

Fig. 7: Determining Air Permeability of Core Plug by NDP-605 NanoDarcy Permeameter

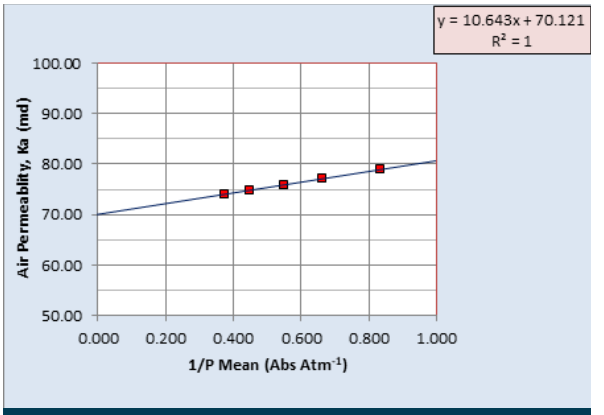


Fig. 8: Air Permeability vs. Inverse of Mean Pressure.

Table 2: The Porosity and Permeability of the Core Plugs

Core plugs	Effective Porosity (%)	Air Permeability (md)	Theoretical Liquid Permeabilty(md)
1	12.56	49.27	41.93
2	20.93	134.87	120.40
3	16.59	80.76	70.12
4	17.88	39.75	31.81
5	18.83	70.75	63.45
6	19.99	65.60	54.19
7	17.68	42.75	33.60
8	16.24	68.41	57.84

2.5 Low Salinity Brine Flooding

The core flooding of the above core plugs was done using Ruska Positive Displacement Pump (Fig. 9) at an injection rate of 112 cc/hour using different low-salinity brine. The salinity of the injection brines was reduced up to 14% from the reservoir brine salinity (1404 ppm). Here, the volume displaced by the movement of the plunger of the pump is expressed on a linear scale calibrated in a cubic centimeter (cc), where the flow rate of the injected liquid is adjusted by adjusting its different levers. In this experiment, more than 15 Pore Volume (PV) oil and

brine were flooded in each of the core plugs. During the flooding, produced brine and oil were collected and measured carefully for further study (Fig. 10).

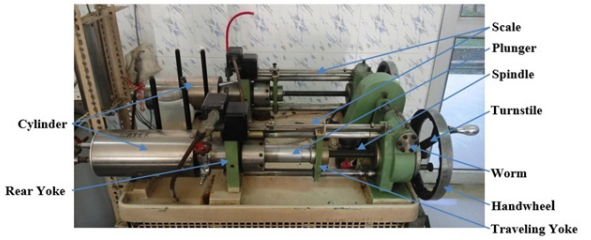


Fig. 9: Ruska Positive Displacement Pump

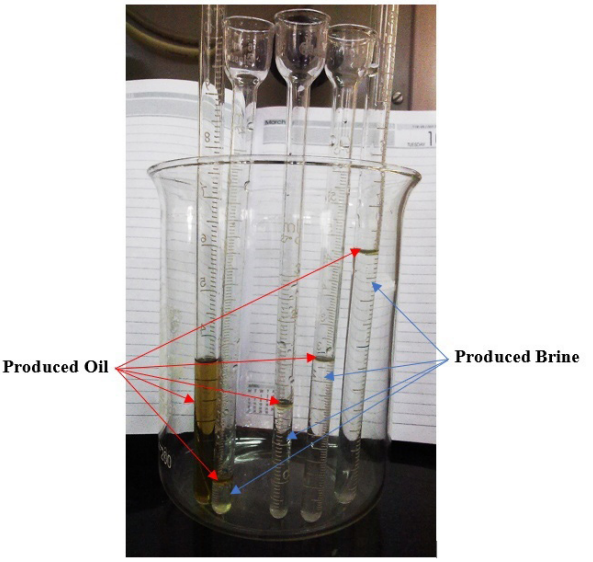


Fig. 10: Produced Brine and Oil during Core Flooding

2.6 Study of Migrated Fines

During the core flooding, some fines were observed in the produced effluent brine, which was migrated along with the flowing brine. In this study, the migrated fines were separated from the produced brine using filter paper. The filter paper containing the fines was dried for a sufficient time. The dry fine sediments were then collected and analyzed under Scanning Electron Microscope (SEM). Fig. 11-12 shows the SEM Photomicrograph of the migrated fine sediments.

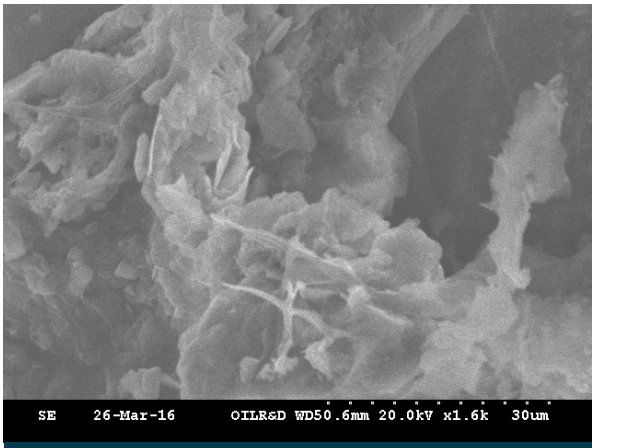


Fig. 11: SEM Photomicrograph of Migrated Fines showing Illite

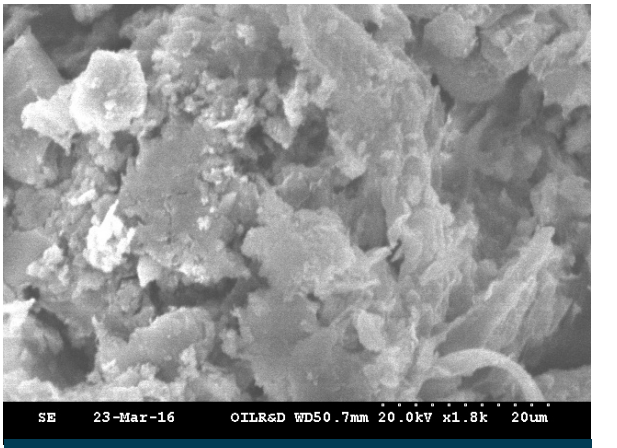


Fig. 12: SEM Photomicrograph of Migrated Fines showing Mixed-layer

2.7 Determination of Porosity and Permeability of the Flooded Core Plugs

For determining the alteration of porosity and permeability during LSW, all the flooded core plugs were cleaned and dried properly using the methods mentioned above. The porosity and the permeability of the core plugs were then determined again using the same porosimeter and permeameter. The porosity and the permeability of the core plugs after flooding are given in Table 3.

Table 3: The Porosity and Permeability of the Flooded Core Plugs

Core plugs	Effective Porosity (%)	Air Permeability (md)	Theoretical Liquid Permeability(md)
1	11.46	47.50	38.46
2	19.54	128.00	112.10
3	16.31	80.75	67.96
4	17.14	37.68	30.11
5	18.59	70.13	63.28
6	17.90	62.46	53.78
7	16.39	38.15	32.35
8	15.12	66.39	55.42

3. Results and Discussion

The rate of In-Situ Clay-Induced formation damage depends on the Physico-chemical factors that control the clays and the properties of the rock matrix surface. Mainly, the clay and mineralogy of the rock matrix, injection fluid chemistry, micromorphology of the reservoir, and crude oil composition govern the extent of the damage in the formation. Therefore, an analysis of the three main elements of the COBR system (Crude Oil, Brine, and Rock) has been done before the study of formation damage and its effects on oil recovery during LSW in the Tipam Reservoir Sandstone of Upper Assam Basin in the depth range 2853.00 m-2973.00 m.

The petrographic analysis (SEM, XRD, and Thin Section) shows that Smectite, Illite, and Kaolinite clays are present in the reservoir rock of the study area along with Mica and Plagioclase Feldspar (Fig. 1-4). As mentioned above, migration of those clays occurs during the injection of low-salinity brine into the formation. In addition to the direct migration of Kaolinite and Illite, Swelling-Induced Migration of clays & Mica and swelling of the Smectite occur during the LSW. This In-Situ migration and swelling of clays can reduce the rock permeability by blocking some of the pore throats. However, the migrated and swelling clays can improve the oil recovery by alteration of rock wettability, reduction of oil-brine IFT, and Improving Sweep Efficiency, as mentioned earlier. Also, the permeability decline may improve the water flood performance by increasing the time of water breakthrough and reducing the water cut (Zeinijahromi *et al*. 2011). The presence of the Plagioclase Feldspar in the rock indicates that the Tipam Reservoir Sandstone is a good candidate for LSW (Hughes *et al*. 2012).

Earlier studies have shown that the substitution of different ions takes place in the lamellae of the clays, which can cause unbalanced internal negative charges in the lamellae (Bathija 2009). These unbalanced negative charges are compensated by the adsorption of oppositely charged ions on the external surfaces of the clays from the surrounding brine (Worden and Morad 2003). It is found that clay minerals can exchange the cations adsorbed on their external surfaces and between the layers of the clay structure, which is called Cation Exchange Capacity (Hamilton 2009). As mentioned earlier, Smectite, Illite, and Kaolinite have high CEC, which can increase the pH of the brine close to their surfaces (Austad *et al*. 2010; Hughes *et al*. 2010). As a result, more clays can be released from the pores' inner wall and then migrate along with the injected low-salinity brine. These migrated clays can block some of the pore throats in the rock resulting in permeability reduction. It is also found that the high pH of the brine phase results in the improvement of water-wetness of the reservoir rock through the desorption of organic materials from the clay surfaces that improve the oil recovery (McGuire *et al*. 2005; RezaeiDoust *et al*. 2010). Additionally, the reaction of acid compounds of crude oil occurs in the high pH environment that generates some surfactant In-Situ (Boussour 2009). These surfactants can reduce the Interfacial Tension (IFT) between the reservoir oil & brine, which further reduces the Residual Oil Saturation in the reservoir. If the pH of the brine increases to above 9 in a petroleum reservoir, the flooding process would be equivalent to an Alkaline Flood (McGuire *et al*. 2005).

The analysis of the formation fluids shows that polar compounds (resin and asphaltene) and divalent cations (Ca^{2+} and Mg^{2+}) are present in the crude oil and formation brine, respectively. It is observed that polar compound adsorption occurs on the clay surfaces when there is direct contact between the oil phase and the rock containing clays. The factors that affect this adsorption on clay surfaces include pH, type of clays, and their exchangeable cations (Clementz 1976; Czarnecka & Gillott 1980; Austad *et al*. 2010). The adsorption of the polar compounds of oil on the clay surfaces occurs mainly by Ligand Bridging and Cation Exchange (Lager *et al*. 2008b). In Ligand Bridging, polar compounds adsorb on the clay surface by multivalent cations (Ca^{2+} and Mg^{2+}), whereas Cation Exchange involves the direct adsorption of polar compounds on

the clay surface by displacing the cations. During LSW, when the clay migration occurs, oil droplets attached to the clay surfaces also move along with the injection fluid, which improves the oil recovery. Also, the high Acid Number of crude oil (0.58) can shift the rock wettability to more water-wet condition through the In-Situ generation of some surfactants during LSW as observed in the earlier studies (Ehrlich *et al*. 1974; Ehrlich & Wygal 1977). This wettability modification can further contribute to the additional recovery of oil.

Analysis of the migrated fines shows that Illite and Mixed-layer are migrated along with the injected low salinity brine during core flooding. Earlier studies also observed Kaolinite migration in the study area during LSW in a core flooding experiment (Medhi 2018). This indicates that Kaolinite, Illite, and Mixed-layer migration occurs in the area under study during LSW, which can improve the recovery of oil along with a reduction of permeability of the core plugs.

The routine core analysis of the prepared core plugs shows that the Porosity and the Air Permeability are in the range of 12.56% - 20.93% and 39.75 md-134.87 md respectively (Table 2). From the Klinkenberg Effect study, the Theoretical Liquid Permeability is found to vary between 31.81 md and 120.40 md. The low salinity core flooding experiment shows that an additional 06.20% of Original Oil In Place (OOIP) oil recovery is possible above the core flooding using reservoir brine. After the core flooding, the Porosity and the Air Permeability of the flooded core plugs are in the range of 11.46%-19.54% and 37.68 md-128 md respectively (Table 3). The Theoretical Liquid Permeability varies between 30.11 md and 112.10 md. It is seen that both the Porosity and the Permeability of the core plugs are reduced after the LSW, which is due to the migration of Illite & Mixed-layer, and swelling of Smectite.

The main finding of the results of this study is the In-Situ Clay-Induced Formation Damage that occurs in Tipam Reservoir Sandstone of Upper Assam Basin during Low Salinity Waterflooding. Reduction of the Theoretical Liquid Permeability from 31.81 md - 120.40 md to 30.11 md - 112.10 md occurs due to the plugging of pore throats by some mechanical and chemical processes like migration of Kaolinite, Illite & Mixed-layer and swelling of Smectite clays. This plugging can increase the oil recovery by improving the Sweep Efficiency. The formation damage caused by Fine Migration and Clay Swelling can improve Sweep Efficiency during LSW by altering rock wettability towards more water-wet conditions, reducing oil-brine interfacial tension, and blocking some of the pore throats of the reservoir rock. The wettability modification and IFT reduction promote the movement of injection water more efficiently in the reservoir thereby improving the Sweep Efficiency. Additionally, the reduction in permeability due to the blockage of pore throats can redirect the injected water toward the previously un-swept regions, ensuring better coverage of the reservoir and improved oil recovery. Moreover, the permeability decline in the Swept Zone can improve the LSW performance by increasing the Time of Breakthrough of the injected low salinity brine and reducing the water cut.

These findings significantly enhance the existing knowledge by comprehensively elucidating the complex mechanisms underlying Clay-Induced Formation Damage and its impacts on oil recovery during LSW. By integrating petrographic analysis of rock, reservoir fluid characterization, and core flooding experiments, the study reveals the complex interactions among clays, brine, and crude oil. Additionally, the study demonstrates the potential of LSW to formation damage while enhancing oil recovery, offering valuable insights for optimizing reservoir management strategies in sandstone reservoirs, particularly in the context of the Tipam Reservoir Sandstone of Upper Assam Basin.

However, the challenges encountered in this study include accurately characterizing the complex physico-chemical interactions among clays, crude oil, injection brine, and formation brine. Additionally, quantifying the extent of damage and its impact on reservoir characteristics requires sophisticated analytical techniques. Moreover, replicating reservoir conditions in laboratory experiments poses logistical and technical challenges. Addressing these limitations is crucial for advancing understanding and optimizing recovery strategies during LSW in Sandstone Reservoirs.

4. Conclusion

The In-Situ Clay-Induced Formation Damage occurs in the area under study during Low Salinity Water flooding by the migration and swelling of Kaolinite, Illite, Smectite, and Mixed-layer. However, this formation damage can improve the oil recovery efficiency by Improving the Sweep Efficiency, increasing the water breakthrough time, and reducing the water cut. The migrated clays can also increase the recovery of oil through wettability modification and oil-brine IFT reduction in the Tipam Reservoir Sandstone (depth range 2853.00 m-2973.00 m) of Upper Assam Basin.

Acknowledgements

The author is thankful to the Oil and Natural Gas Corporation Limited (ONGCL), Jorhat, Assam,for providing the Rock Samples and Formation Brine & some other relevant data for this study. The author also wishes to thank Oil India Limited (OIL), Duliajan, Assam, Department of Petroleum Engineering, Department of Petroleum Technology, and Department of Applied Geology for providing the required laboratory facilities for this work.

References

Al-Hetari, N.M., 2017. Formation Damage in Oil and Natural Gas Reservoirs. Selected Topics in Petroleum Engineering, PET447E, Istanbul Technical University, Maslak, Istanbul.

API RP 40, 1998. Recommended Practices for Core Analysis. Second Edition. American Petroleum Institute, USA.

Austad, T., A. Rezaeidoust, and T. Puntervold, 2010. Chemical Mechanism of Low Salinity Water Flooding in Sandstone Reservoirs. SPE-129767-PP, Presented at the 2010 SPE Improved Oil Recovery Symposium, Tulsa, Oklahoma, USA. DOI: 10.2523/129767-MS

Bathija, A.P., 2009. Elastic Properties of Clays. Thesis, Colorado School of Mines, Colorado, USA.

Bennion, D. B., F. B. Thomas, D. W. Bennion, and R. F. Beitz, 1995. Mechanisms of Formation Damage and Permeability Impairment Associated with the Drilling, Completion, and Production of Low Gravity Oil Reservoir. SPE-30320-MS, Presented at the SPE International Heavy Oil Symposium, Calgary, Alberta, Canada. DOI: 10.2523/30320-MS

Boussour, S., M. Cissokho, P. Cordier, H. Bertin, and G. Hamon, 2009. Oil Recovery by Low-Salinity Brine Injection: Laboratory Results on Outcrop and Reservoir Cores. SPE-124277-MS, Presented at the SPE Technical Conference and Exhibition, New Orleans, Louisiana. DOI: 10.2118/124277-MS

Brahma, D. G., H. Talukdar, S. Handique, S. Das, S. R. Nikhar, and T. Pegu, 2017. A Study on Reservoir Characteristics. B.Tech. Project Report, Dibrugarh University, Assam, India.

Bruin, W. J., 2012. Simulation of Geochemical Processes during Low Salinity Water Flooding by Coupling Multiphase Buckley-Leverett Flow to the Geochemical Package PHREEQC, M.Sc. Thesis, Delft University of Technology, Netherlands.

Civan, F., 2007. Temperature effect on power for particle detachment from pore wall described by an Arrhenius-type equation. *Transp. Porous Med.,* 67(2): 329-334. DOI: 10.1007/s11242-006-9005-3

Civan, F., 2010. Non-isothermal permeability impairment by fines migration and deposition in porous media including dispersive transport. *Transp. Porous Med.,* 85(1): 233-258. DOI: 10.1007/s11242-010-9557-0

Civan, F., 2015. Reservoir Formation Damage: Fundamentals, Modeling, Assessment, and Mitigation. Third Edition, Gulf Professional Publishing, Houston, Texas

Clementz, D. M., 1976. Interaction of Petroleum Heavy Ends with Montmorillonite. *Clays and Clay Minerals*, 24: 312-319. DOI: 10.1346/CCMN.1976.0240607

Czarnecka, E., and J. E. Gillott, 1980. Formation and Characterization of Clay Complexes with Bitumen from Athabasca Oil Sand. *Clay and Clay Minerals*,28(3):197-203. DOI: 10.1346/CCMN.1980.0280305

Economides, M. J., A. D. Hill, and C. Ehlig-Economides, 1994. Petroleum Production Systems, Englewood Cliffs, N.J.: PTR Prentice Hall, New Jersey, U.S.A.

Ehrlich, R. and R. J. Wygal, 1977. Interrelation of Crude Oil and Rock Properties with the Recovery of Oil by Caustic Waterflooding.*SPE Journal,*17(04):263-270. DOI: 10.2118/5830-PA

Ehrlich, R., H. H. Hasiba, and P. Raimondi, 1974. Alkaline Water flooding for Wettability Alteration – Evaluating a Potential Field Application. *Journal of Petroleum Technology*, 26:1335-1343. DOI: 10.2118/4905-PA

Ezenweichu, C. L., and O. D. Laditan, 2015. The Causes, Effects and Minimization of Formation Damage in Horizontal Wells. *Petroleum & Coal*, 57(2):169-184.

Faergestad, I., 2016. The Defining Series: Formation Damage, Oilfield Review, Schlumberger.

Fogden, A., 2012. Removal of crude oil from kaolinite by water flushing at varying salinity and pH.*Colloid Surf. A-Physicochem. Eng. Asp.*, 402:13–23. DOI: 10.1016/j.colsurfa.2012.03.005

Hamilton, P. J., 2009. A Review of Radiometric Dating Techniques for Clay Mineral Cements in Sandstones. In: Clay Mineral Cements in Sandstones, Worden R.H. and Morad S. (Editor), Special Publication, 253-287. DOI: 10.1002/9781444304336.ch12

He, J., I. M. Mohamed, and H. A Nasr-El-Din, 2013. Potential Formation Damage Due to Preparing HCl Acids Using Seawater. *Canadian Energy Technology and Innovation Journal*, 1(2):56-65.

He, J., I. M. Mohamed, and H. A. Nasr-El-Din, 2012. Mitigation of Calcium Sulfate Scale Formation when Seawater is used to Prepare HCL-Based Acids.SPE-151143-MS, Presented at the SPE International Symposium and Exhibition on Formation Damage Control, Lafayette, Louisiana, USA. DOI: 10.2118/151143-MS

Hibbeler, J., T. Garcia, and N. Chavez, 2003. An Integrated Long-Term Solution for Migratory Fines Damage, SPE-81017-MS, Presented at the SPE Latin American and Caribbean Petroleum Engineering Conference, Port-of-Spain, Trinidad, and Tobago.DOI: 10.2118/81017-MS

Huang, T., J. B. Crews, and J. R. Willingham, 2008. Using Nanoparticle Technology to Control Fine Migration, SPE-115384-MS, Presented at the SPE Annual Technical Conference and Exhibition, Denver, Colorado, USA.DOI: 10.2118/115384-MS

Hughes, D., S. Larsen, and R. Wright, 2010. Review of Low Salinity Water Flooding. A10DEC015A, Department of Energy and Climate Change.

Hughes, D., S. Law, and G. Pitt, 2012. Low Salinity EOR "State of Play" Review. A12DEC056A, Department of Energy and Climate Change, Scotland.

Kalfayan, L. J., and D. R. Watkins, 1990. A new Method for Stabilizing Fines and Controlling Dissolution during Sandstone Acidizing. SPE-20076-MS, Presented at the SPE California Regional Meeting, Ventura, California. DOI: 10.2523/20076-MS

Lager, A., I. R. Collins, G. R. Jerauld, P. L. Mcguire, and K. Webb, (2006-8). BP Low Salinity Patent Application (Hydrocarbon Recovery Process), PCT/GB2007/003337 - WO 2008/029124 A1.

Lager, A., K. J. Webb, C. J. J. Black, M. Singleton, and K. S. Sorbie, 2008a. Low Salinity Oil Recovery-An Experimental Investigation. *Petrophysics*, 49(1):28-35.

Lager, A., K. J. Webb, I. R. Collins, and D. M. Richmond, 2008b. LoSal Enhanced Oil Recovery: Evidence of Enhanced Oil Recovery at the Reservoir Scale, SPE-113976-MS, Presented at the 2008 SPE/DOE Improved Oil Recovery Symposium, Tulsa, Oklahoma, USA. DOI: 10.2118/113976-MS

McGuire, P. L., J. R. Chatman, F. K. Paskvan, D. M. Sommer, and F. H. Carini, 2005. Low Salinity Oil Recovery: An Exciting New EOR Opportunity for Alaska's North Slope, SPE-93903-MS, Presented at the SPE Western Regional Meeting, Irvine, California, U.S.A. DOI: 10.2523/93903-MS

Medhi, N., 2018. A study on Fine's Migration during Low Salinity Water flooding in Geleki Oilfield of Upper Assam Basin. *Journal of Applied Geochemistry*, 20(3):344-351.

Medhi, N., 2018. A study on Fine's Migration during Low Salinity Water flooding in Geleki Oilfield of Upper Assam Basin. Journal of Applied Geochemistry, 20(3):344-351.

Medhi, N., and M. Das, 2015. A Study on the Low Salinity Water flooding (LSW) in a part of Depleted Oil Field of Upper Assam Basin. *Journal of Petroleum Engineering and Technology,* 5(3):1-9.

Mohan, K. K., R. N. Vaidya, M. G. Reed, and H. S. Fogler, 1993. Water sensitivity of sandstones containing swelling and non-swelling clays. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 73(1993):237-254. DOI: 10.1016/0927-7757(93)80019-B

Portier, S., L. André, F. D. Vuataz, 2007. Review on chemical stimulation techniques in oil industry and applications to geothermal systems. Technical Report, Engine, work package 4, 32.

Radwan, A. E., A. M. Abudeif, M. M. Attia, and M. A. Mahmoud, 2019. Development of formation

damage diagnosis workfow, application on HammamFaraun reservoir: a case study, Gulf of Suez, Egypt. *Journal of African Earth Sciences,* 153:42–53.
DOI: 10.1016/j.jafrearsci.2019.02.012

RezaeiDoust, A., T. Puntervold, and T. Austad, 2010. A discussion of the Low Salinity EOR potential for a North Sea Sandstone Field, SPE-134459-MS,Presented at the SPE Annual Technical Conference and Exhibition, Florence, Italy.
DOI: 10.2118/134459-MS

RezaeiDoust, A., T. Puntervold, S. Strand, and T. Austad, 2009. Smart Water as Wettability Modifier in Carbonate and Sandstone: A Discussion of Similarities/Differences in the Chemical Mechanisms. *Energy & Fuels,* 23(9):4479-4485.
DOI: 10.1021/ef900185q

Rosenbrand, E., C. Kjoller, J. F. Riis, F. Kets, and I. L. Fabricius, 2015. Different Effects of Temperature and Salinity on Permeability Reduction by Fines Migration in Berea Sandstone. *Geothermics,* 53:225-235.
DOI: 10.1016/j.geothermics.2014.06.004

Scott, P., and M. Davies, 1993. Souring of new Irian Jaya wells traced to indigenousbacteria. *Oil and Gas Journal,* 91:47-49.

Shibulal, B., Al-Bahry, S. N., Al-Wahaibi, Y. M., Elshafie, A. E., Al-Bemani, A. S., and Joshi, S. J., 2014. Microbial enhanced heavy oil recovery by the aid of inhabitant spore-forming bacteria: an insight review. *The Scientific World Journal*, 2014: 309159.
DOI: 10.1155/2014/309159
PMID:24550702 PMCID:PMC3914512

TTang, G. Q., and N. R. Morrow, 1999. Oil Recovery by Water flooding and Imbibitions – Invading brine Cat ionValency and Salinity, SCA 9911, Department of Chemical and Petroleum Engineering, University of Wyoming.

Tang, G. Q., and N. R. Morrow, 1999a. Influence of Brine Composition and Fines Migration on Crude Oil/Brine/Rock Interactions and Oil Recovery. *Journal of Petroleum Science and Engineering,* 24:99-111.
DOI: 10.1016/S0920-4105(99)00034-0

Vaidya, R. N., and H. S. Fogler, 1990. Formation Damage due to Colloidally Induced Fines Migration. *Colloids and Surfaces,* 50:215-229.
DOI:10.1016/0166-6622(90)80265-6

WWilson, M. J., L. Wilson, and I. Patey, 2014. The influence of individual clay minerals on formation damage of reservoir sandstones: a critical review with some new insights. *Clay Minerals,*49:147-164.
DOI: 10.1180/claymin.2014.049.2.02

Worden, R. H., and S. Morad, 2003. Clay Mineral Cements in Sandstones. Published for the International Association of Sedimentologists, Blackwell Pub., Oxford.

Zeinijahromi, A., P. Lemon, and P. Bedrikovetsky, 2011. Effects of Induced Migration of Fines on Water Cut during Waterflooding.SPE-139239-MS, Presented at the SPE Middle East Oil and Gas Show and Conference, Manama, Bahrain.
DOI: 10.2118/139239-MS

Zhou, X., F. B. Thomas, and R. G. Moore, 1996. Modeling of Solid Precipitation from Reservoir Fluid.*Journal of Canadian Petroleum Technology,* 35(10).
DOI: 10.2118/96-10-03
PMID: 20507814

Zhou, Z. J., W. O. Gunter, and R. G. Jonasson, 1995. Controlling Formation Damage Using Clay Stabilizers: A Review. PETSOC-95-71, Presented at the Annual Technical Meeting, Calgary, Alberta.
DOI: 10.2118/95-71

Effect of Various Organic Priming Treatments on the Performance of Bittergourd (*Momordica Charantia* L)

Nisha Karki[1*], Rekha Bhattarai[2], Eliza Ghimire[1] and Kapil Khanal[3]

[1]Institute of Agriculture and Animal Science (IAAS), Mahindra Ratna Multiple Campus, Ilam.
[2]Kathmandu University
[3]Agriculture and Forestry University

***CORRESPONDING AUTHOR:**
Nisha Karki
Email:karkinisha3939@gmail.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI :
https://doi.org/10.3126/njst.v21i2.67164

Date of Submission: Mar 16, 2023

Date of Acceptance: Jan 2, 2024

ABSTRACT

A field experiment was carried out in Godak-10, Ilam, during the cropping season of February to July 2021, to investigate the effect of organic seed priming treatments on bitter gourd growth performance. Hard seed coat of bitter gourd puts mechanical restriction on embryo development and it is the reason for poor seedling problems. Seed priming is the method not only to improve germinations, vigour and uniform emergence of seedlings in field conditions but also to improve crop establishment and ultimately enhance the yield. The experiment was done in a double-factor randomized complete block design with 3 replications and 8 treatments. Three varieties of bittergourd viz. Palee, Asmita (Syngenta), and local, were assigned as the first factor. Whereas, priming treatments with cow milk and water 1:1, cow urine and water 1:1, cow dung slurry and water 1:2, water and control (no priming) were assigned as second factor. Parameters such as seed germination percentage (83.66%), day to emergence (9 days), seedling's stem diameter (3.12 mm), shoot weight (1.8 gm), shoot length (11.5 cm), root length (14.4 cm), plant height 20 days after transplanting (67.4 cm) and 40 days after transplanting (125 cm), number of leaves 20 days after transplanting (21) and tendrils 20 days after transplanting (14), day to first flowering (33 days), day to first fruit set (39 days) and yield (3.02 kg/plant) were significantly higher in the variety Palee. Seed germination percentage (71.55%), seedling stem diameter (3.05 mm) were significantly higher with the priming treatment of cow urine and water 1:1, but other parameters, the priming treatment was significantly at par with all treatments except control. The study indicated that presoaking of bittergourd seeds in organic priming treatment, i.e., cow urine and water 1:1, could render better growth performance.

Keywords: Bittergourd, Cow dung slurry, Cow milk, Cow urine, Organic seed priming

1. Introduction

The bitter gourd (*Momordica charantia* L), which belongs to the cucurbitaceae family is one of the most popular vegetables in Southeast Asia as well in Nepal. The name "bitter-gourd" might also refer to bitter-melon, balsam pear, or karela, depending on geographic location (Palada & Chang 2003). Bittergourd, due to its medicinal properties, is well-liked for treating numerous illnesses like blood disorders, asthma, rheumatism, and diabetes (Tania *et al*. 2019). It is also recommended for treating other illnesses like anemia, diarrhea, dysentery, bronchitis, ulcer, cholera, and gonorrhea. The plant also has hypoglycemic, anti-tumor, anti-carcinogenic, anti-parasitic, anti-fertility, and anti-viral properties because of the numerous of biologically active plant chemicals it contains, such as alkaloids, proteins, triterpenes, and flavonoids (Sowmya *et al*. 2018).

Due to the presence of a hard seed coat all the bitter gourd genotypes show a problem with germination (Sowmya *et al*. 2018). Therefore seeds must undergo pre-sowing treatments despite having excellent germinability (Saleem *et al*. 2014). As compared to chemicals, organic priming (cow products) is cost-effective, environmentally friendly and easy to use by farmers (Kumhar *et al.* 2018). Poor seedling germination hampered emergence, and a sluggish growth rate due to a seed-coated embryo is the main issue with the bitter gourd's restricted yield (Khan *et al*. 2019). Cow products are superior to chemicals those increase soil fertility, are affordable, have a positive impact on subsequent crops, are non-polluting and harmless to beneficial soil bacteria, and require less special care and precautions (Kumhar *et al*. 2018).

Growth-promoting hormones are present in cow dung (Shinde & Malshe 2015). Growth regulators, nutrients, and trace elements are physiologically active chemicals that are present in cow urine (Tania *et al*. 2019).There is a paucity of knowledge regarding the impact of hormones from natural sources, such as fresh cow milk, on the germination of seeds of agroforestry tree species (Adelani & Bello 2016). Immerse the seeds in raw cow's milk for 24 hours to promote good germination and yield before planting (Sridhar & Kumar 2013). Seed germination, seedling growth, and bitter gourd yield can all be improved by hydro-priming (Malika & Rawat 2020). Hydropriming is an environmentally friendly, straightforward, and cost-effective method of seed priming (Tania *et al*. 2019). Cow urine, which contains nutrients like potassium and is known to have positive effects on germination, growth components such as plant height, number of leaves, and leaf area, and yield components such as number of grains, number of tillers, grain weight, and yield of crops, is a cheap input and easily available to rural farmers (Tiwari *et al*. 2018). Most of the modern seed priming involves chemical and hormonal priming. These modern priming are not economically affordable, easy to handle or environmentally friendly. Research related to organic priming is comparatively lower than that related to chemical priming in Nepal.

Hence, the objective of this research was to analyze the effect of various organic seed priming treatments on the growth performance of different genotypes of bittergourd.

2. Materials And Methods

The experiment was carried out in Godak Village in Ilam district of province number 1. It is Located at 26º 54' 42.12'' north latitude, 87º55'12.7'' east longitude, 849.4 masl. Ilam experiences fridge winters, pleasant summers, and hazy monsoons. The experiement was laid out in a Random Complete Block Design with a two-factor factorial with replications and 8 treatments namely factor 1 (Variety) and factor 2 (Priming Treatments).

Factor 1: Varieties of Bittergourd i.e., V1: Palee, V2: Asmita (Syngenta), and V3:Local.

Factor 2: Organic priming treatments (T1: cow milk and water at 1:1 ratio, T2 : cow urine and water at 1:1 ratio, T3 cow dung slurry and water at 1:2, T4 : water, T5: control (no seed priming).

Three tunnels were constructed, each measuring 2 meters in length and 1 meter in breadth. The distance between each tunnel was 0.5 meters. A polythene bag was used of 12 cm long by 6.5 cm wide and 100 gauge thick. A polythene bag was filled with the media after the soil, sand, and Farm Yard Manure completely mixed in a 2:1:1 ratio. For the research projects, 1,125 polythene bags 375 in each tunnel were utilized. A 1:1 ratio of raw cow's milk to tap water was employed. Clean tap water and cow dung were used in a ratio of 1:2. During hydro priming, clean tap water was used. After that the primed seeds were kept in the shade to dry. After drying, seeds were sown in polythene bags. Seeds were sown in each bag and were watered daily with the help of a

sprinkler can. Three seedlings of 25 old were selected and transplanted in each treatment plots in the field. The total number of plants transplanted in the field was 135 (45 plants from each replication and 3 from each treatment). Plant - to- Plant distance was maintained at 1.5 meter in the field.

Initially, bullock cards were used to plough the field. A pit of 30 cm by 30 cm was dug, and 4 kg of well-decomposed FYM was placed inside the pit. Irrigation and other interrelated tasks were completed after transplantation. On the 25 and 40 DAT, two-hand weeding was carried out. During the growing period, yellowing of lower leaves was seen; Cow urine mixed with water (1:5) was sprayed through a sprinkler can in the entire plants of the experimental field. No other issues related to insect pests were faced. All collected data were compiled and entered in Microsoft Excel (2007). Collected data were set in software named Rstat version 1.4.1106 and packages used in R (Agricolae version 1.3-5, readxl version 1.3.1, and rstatix version 0.7.0), DMRT (Duncan Multiple Range Test) for mean separation at a 5% level of significance was done.

3. Results and Discussion

3.1 Germination percentage and Day to Emergence

A significant result was recorded for the germination percentage under the different pre-sowing treatments. Among the three varieties, the Palee variety showed the significantly highest germination percentage (83.66%) followed by Asmita (56.33%) and the Local variety (40.73%). Likewise, among all the priming treatments, cow urine and water at 1:1-treated seed showed the highest germination percentage (71.55%), which is significantly at par with Tap water - treated seeds (69.55%)and significantly different from other priming treatments.

Among three varieties, Palee variety showed significantly lower days to emergence followed by Asmita and Local variety. Analysis of variance reveled significant differences between the priming treatments for the germination percentage and day to emergence (Table 1).

Interaction effect of different variety and priming treatment on day to emergence of bittergourd seedlings shows palee variety with cow dung slurry treatment showed lesser days to emergence while local variety with control (no seed priming) showed more days to emergence (Table 2).

Table 1: Effect of different variety and priming treatment on germination percentage (20 days after sowing) and day to emergence of bittergourd seedlings.

Treatment / Variety	Germination Percentage	Day to Emergence
Palee	83.6±2.60 ^{a}	9 ±0.44^{c}
Asmita	56.33±4.22^{b}	11±0.64^{b}
Local	40.73± 4.04^{c}	13±0.78^{a}
LSD 0.05	8.21***	0.64***
Priming Treatment		
Cow Milk (1:1)	63.00±6.82^{ab}	10±0.687^{b}
Cow Urine(1:1)	71.55±5.10^{a}	10±0.455^{b}
Cow Dung Slurry(1:2)	55.77±7.92 ^{b}	10±0.619^{b}
Tap water	69.55±6.73^{a}	10±0.588^{b}
control	41.33±10.5^{c}	15±0.986^{a}
mean	60.61	11
LSD0.05	0.83***	0.89***
CV%	1.42	7.62
Interaction(Variety * PrimingTreatment) LSD 0.05	1.44 ^{ns}	

Note: Means followed by common letter(s) in the superscript within a column are non significantly different at

5% by LSD, CV = Coefficient of variation, LSD = Least significant difference, SEM (±) = Standard error of mean difference, * significant at 5%, ** significant at 1%, *** significant at 0.1%, NS = non-significant.

Table 2: Interaction effect of different variety and priming treatment on day to emergence of bittergourd seedlings.

Treatment/Variety	Palee	Asmita	Local
Cow Milk soaking (1:1)	8.33 ±0.33^{gh}	10.33±1.20^{def}	12.33±0.33^{c}
Cow Urine soaking (1:1)	9.00± 0.57^{fgh}	9.67±0.33^{efg}	11.67±0.33^{cd}
Cow Dung Slurry (1:2)	8.00±0^{h}	11.00±0.58^{cde}	11.67±0.67^{cd}
Tap water	8.33±0.33^{gh}	10.00±0.58^{ef}	12.00±0.58^{c}
control	12.33±0.33^{c}	15.67±0.33^{b}	19.00±0.578^{a}
mean	11.09		
LSD 0.05	1.43*		
CV%	7.62		

Note: Means followed by common letter(s) in the superscript within a column are non significantly different at 5% by LSD, CV = Coefficient of variation, LSD = Least significant difference, SEM (±) = Standard error of mean difference,* significant at 5%, ** significant at 1%, *** significant at 0.1%, NS = non-significant.

3.2 Stem Diameter

Among three varieties Palee showed the significantly thicker stem diameter (3.12 mm) followed by Asmita and Local variety. Likewise, among all the priming treatments, cow urine and water at 1:1-treated seed showed the maximum stem diameter (3.05 mm), which is at par with cow milk (2.83 mm), cow dung slurry (3.03 mm), tap water (2.70 mm). The use all priming techniques recorded significantly higher stem diameter from the control (1.99 mm). Whereas, the interaction effect showed no significant influence on stem diameter. Analysis of variance revealed significant differences among the priming treatments for the stem diameter (Table 3).

Table 3: Effect of different Variety and Priming treatment on Stem diameter of Bittergourd seedlings (20 Days after Sowing)

Treatment / Variety	Stem diameter(mm)
Palee	3.12±0.08^{a}
Asmita	2.72±0.159^{b}
Local	2.32±0.16^{c}
LSD 0.05	0.25***
Priming Treatment	
Cow Milk (1:1)	2.83±0.14^{a}
Cow Urine (1:1)	3.05±0.09^{a}
Cow Dung Slurry (1:2)	3.03±0.11^{a}
Tap water	2.70±0.12^{a}
control	1.99±0.29^{b}
mean	2.76
LSD 0.05	0.32***
CV%	12.54
Interaction (Variety and Priming Treatment) LSD 0.05	0.57^{ns}

Note: Means followed by common letter(s) in the superscript within a column are significantly different at 5% by LSD, CV = Coefficient of variation, LSD = Least significant difference, SEM (±) = Standard error of mean difference, * significant at 5%, ** significant at 1%, *** significant at 0.1%, NS = non-significant.

3.3 Shoot Weight and Root Weight

Among the three varieties, the Palee variety showed the maximum shoot weight (1.8 gram), which was at par with the Asmita variety, followed by the Local variety. Meanwhile, the Asmita variety showed the maximum root weight (0.40 gram) which is at par with the Palee variety. Likewise, the Interaction effect showed none significantly influence shoot weight and root weight. Analysis of variance revealed significant difference among the priming treatments for the shoot and root weight (Table 4).

Table 4: Effect of different variety and priming treatment on shoot and root weight of bittergourd seedlings (20 days after sowing).

Treatment / Variety	Shoot Weight (gram)	Root Weight (gram)
Palee	1.8±0.10[a]	0.36±0.05[a]
Asmita	1.71±0.11[a]	0.40±0.06[a]
Local	0.79±0.09[b]	0.11±0.02[b]
LSD 0.05	0.30***	0.11***
Priming Treatment		
Cow Milk (1:1)	1.54±0.20	0.33± 0.09
Cow Urine (1:1)	1.63±0.12	0.33± 0.07
Cow Dung Slurry (1:2)	1.42±0.18	0.27± 0.08
Tap water	1.51±0.28	0.31± 0.08
control	1.07±0.24	0.24± 0.07
mean	1.43	0.29
LSD 0.05	0.39[ns]	0.15[ns]
CV%	26.96	36.16
Interaction (Variety and Priming Treatment)LSD 0.05	1.43[ns]	1.43[ns]

Note: Means followed by common letter(s) in the superscript within a column are non-significantly different at 5% by LSD, CV = Coefficient of variation, LSD = Least significant difference, SEM (±) = Standard error of mean difference, * significant at 5%, ** significant at 1%, *** significant at 0.1%, NS = non-significant.

3.4 Shoot Length and Root Length

Among the three varieties, the Asmita variety showed the maximum shoot and root length which is at par with the Palee variety but significantly longer than the Local variety. There was no significant influence priming treatments on shoot and root length (Table 5)

.

Table 5: Effect of different variety and priming treatment on shoot length and root length of bittergourd seedlings (20 Days After Sowing)

Treatment /Variety	Shoot Length (cm)	Root Length (cm)
Palee	11.5±0.40[a]	14.4±1.27[a]
Asmita	10.5±0.63[a]	13.6±1.35[a]
Local	0.79±0.10[b]	8.16±1.23[b]
LSD0.05	1.31 ***	2.84***
Priming Treatment		
Cow Milk (1:1)	7.62±1.73	13.3±2.09
Cow Urine (1:1)	8.05±1.73	14.7±1.10
Cow Dung Slurry (1:2)	7.96±1.98	11.0±1.95
Tap water	7.37±1.76	11.7±1.72
control	6.93±1.76	9.47±2.20
mean	7.58	12.03
LSD0.05	1.7[ns]	3.67[ns]
CV%	23.20	31.62
Interaction (Variety*Priming Treatment)LSD 0.05	2.94[ns]	6.36[ns]

Note: Means followed by common letter(s) in the superscript within a column arenon significantly different at 5% by LSD, CV = Coefficient of variation, LSD = Least significant difference, SEM (±) = Standard error of mean difference, * significant at 5%, ** significant at 1%, *** significant at 0.1%, NS = non-significant.

3.5 Plant Height

The Palee variety showed the maximum primary branch length (67.4 cm) at twenty days after transplanting and (125 cm) at forty days after transplanting, which is significantly at par with Asmita and significantly different with Local variety. Likewise, among all the priming treatments cow urine and water at 1:1 treated seed showed the maximum stem diameter which is significantly at par with other priming treatments except control. Whereas, the interaction effect showed a non-significant influence on primary branch length (Table 6).

Table 6: Effect of different variety and priming treatment on primary branch length after 20 and 40 Days after Transplant (DAT).

Treatment Variety	Plant Height (cm) 20 DAT	Plant Height (cm) 40 DAT
Palee	67.4±12.0 [a]	125±9.34[a]
Asmita	60.1±10.6 [a]	118.8±8.03[a]
Local	43.4± 6.53[b]	104±4.17[b]
LSD	12.78**	11.56**
Priming Treatment		
Cow Milk (1:1)	57.4±12.0[a]	116.6±8.74[a]
Cow Urine (1:1)	66.3±14.0[a]	125.4±10.1[a]
Cow Dung slurry (1:2)	61.8±15.7[a]	120.30±11.9[a]
Tap water	63.6±14.9[a]	122.34±11.1[a]
control	35.9±7.22[b]	94.21±4.37[b]
mean	57.01	115.7
LSD	16.50**	14.92**
CV%	29.97	13.35
Interaction (Variety*Priming Treatment)LSD0.05	28.57[ns]	13.35[ns]

Note: Means followed by common letter(s) in the superscript within a column are non-significantly different at 5% by LSD, CV = Coefficient of variation, LSD = Least significant difference, SEM (±) = Standard error of mean difference, * significant at 5%, ** significant at 1%, *** significant at 0.1%, NS = non-significant.

3.6 Number of leaves and number of tendrils

The Palee variety had the highest number of leaves and tendrils par with the Asmita but significantly higher than the Local variety. Likewise, among all the priming treatments cow urine and water at 1:1-treated seed showed higher number of leaves and tendrils which was significantly at par with other priming treatments except control. Whereas, the interaction effect showed no significant influence on the number of leaves and tendrils (Table 7).

Table 7: Effect of different variety and priming treatment on number of leaves, number of tendrils of bitter gourd plants 20 Days after Transplant.

Treatment Variety	Number of Leaves	Number of Tendrils
Palee	21±2.12[a]	14 ±1.50[a]
Asmita	21±2.10[a]	10±1.25[b]
Local	16 ±1.22[b]	8± 0.91[c]
LSD	3.14 **	1.71***
Priming Treatment		
Cow Milk (1:1)	20±1.73[b]	13± 1.51[ab]
Cow Urine (1:1)	22±2.90 [a]	14±1.95[a]
Cow Dung Slurry(1:2)	20±2.53[b]	11±1.79[bc]
Tap water	21±2.82[ab]	10±1.52[c]
control	13 ±1.37 [c]	5±0.9[d]
mean	19	11
LSD	4.06**	2.21***
CV%	21.4	20.61
Interaction (Variety*Priming Treatment)LSD	7.03[ns]	3.83 *

Note: Means followed by common letter(s) in the superscript within a column arenone significantly different at 5% by LSD, CV = Coefficient of variation, LSD = Least significant difference, SEM (±) = Standard error of mean difference, * significant at 5%, ** significant at 1%, *** significant at 0.1%, NS = non-significant.

3.7 Day to first flowering and first fruit set

Among the three varieties, the Palee variety showed lower days to first flowering (33 days) and first fruit set (39 days), which is significantly different from the Local Variety. Likewise, among all the priming treatments cow urine and water at 1:1 treated seed showed lower days to first flowering and first fruit set. Whereas, the interaction effect showed no significant influence on Day to First flowering and First fruit set (Table 8).

Table 8: Effect of different variety and priming treatment on day to first flowering, day to first fruit set of bittergourd plant.

Treatment / Variety	Day to first flowering	Day to First fruit set
Palee	33±1.74[b]	39 ±1.59[b]
Asmita	35±2.06[b]	45±1.12 [a]
Local	40±2.23[a]	45±1.60[a]
LSD	3.14***	2.55***
Priming Treatment		
Cow Milk (1:1)	33±1.81[bc]	40±1.43[b]
Cow Urine (1:1)	31±1.87 [c]	40±1.48[b]
Cow Dung slurry (1:2)	36±2.13[b]	42± 1.44[b]
Tap water	34±1.91[bc]	40±1.43 [b]
control	46±2.87[a]	50±2.36[a]
mean	36	43
LSD	3.83***	3.29***
CV%	10.9	7.96
Interaction(Variety*Treatment)LSD 0.05	6.63[ns]	5.71[ns]

3.8 Yield (Fruit weight) kg per plant

Altogether, three harvests were done. Among the three varieties, the Palee variety showed the highest fruit weight per plant (3.02 kg) which is significantly at par with the Asmita variety and local variety. Likewise, among all the priming treatments, cow urine with water at 1:1 treated seed showed the maximum fruit weight kg per plant (2.72 kg) which is significantly at par with other treatments except control. Whereas, the interaction effect showed no ficant influence on the fruit weight per plant.

Table 9: Effect of different variety and priming treatment on fruit weight (yield) up to third harvest of bittergourd plant.

Treatment / Variety	Yield (Fruit weight) kg per plant
Palee	3.02±0.20[a]
Asmita	3.01±0.10[a]
Local	1.26±0.028[b]
LSD	0.31***
Priming Treatment	
Cow Milk (1:1)	2.63±0.33[a]
Cow Urine (1:1)	2.72±0.34[a]
Cow Dung slurry (1:2)	2.57±0.33[a]
GA3 (100)	2.51±0.34[a]
Tap water	2.34±0.33[a]
control	1.90±0.27[b]
mean	2.43
LSD	0.41**
CV%	17.52

Note: Means followed by common letter(s) in the superscript within a column are non significantly different at 5% by LSD, CV = Coefficient of variation, LSD = Least significant difference, SEM (±) = Standard error of mean difference, * significant at 5%, ** significant at 1%, *** significant at 0.1%, NS = non-significant.

4. Discussion

Among the three varieties of bittergourd, the palee variety showed a significantly better growth performance and yield. It might be superior genetic ability of this variety among others in all the priming treatment. Among all the priming treatments, seeds treated with organic

priming treatments showed significant results in terms of growth performance than the control. Cow urine and water at a ratio of 1:1- treated seed showed significant performances in terms of seed germination percentage, lesser days to emergence, and thicker stem diameter; This might be the result of presence of physiologically active substances such as growth regulators, nutrients and trace elements in cow urine.

These findings are also in line with the following findings.

Seeds from all varieties of chickpeas treated with cow urine showed the highest rate of germination in comparison to the control. (Tiwari *et al*. 2018). The treatment (1 kg of cow dung to 2 liter of water) induces seed germination five days earlier than normal sowing in Chilly and controls seed-borne diseases like fruit rot (Karthikeyan *et al*. 2006). Cow milk contains hormones that help to break the mechanical, thermo-dormancy, optical, and physiological of seeds. (Rahman *et al*. 2021). Increased concentrations of growth-promoting chemicals in cow urine may have an impact on seed germination (Tiwari *et al*. 2018).

Chickpea variety treated with organic priming (cow urine) significantly differed from the control in terms of maximum germination (%), seedling length, and fresh weight of chickpea variety seedlings. (Tiwari *et al*. 2018). A two percent urea priming treatment in spring rice produced the longest roots and shoots seven days after sowing (Koirala *et al*. 2019).

Under the agro-climatic circumstances of district Bhimber, Azad Kashmir, palee demonstrated to be the most effective in terms of highest germination and enhancing growth (Saleem *et al*. 2014).

5. Conclusion

Among the tested varieties, the Palee showed the highest growth performance as well the yield of bittergourd followed by the Asmita and Local varieties. It indicated that improved varieties were better than local variety due to their superior genetics abilities.

Among all the priming treatments, cow urine and water at a ratio of 1:1- treated seed showed significant performances in terms of seed germination percentage, shorter days to emergence, and thicker stem diameter. Also, this treatment recorded better performances in terms of growth parameters i.e. plant height, number of leaves and tendrils, lesser days to first flowering, and fruit yield.

Therefore, seed germination, seedling growth performances and yield of bittergourd could be improved through pre-soaking in organic priming cow urine and water at the rate of 1:1 which is cheap, non-toxic, and eco-friendly organic sources. However, such study should be carried out in other crops which have germination problem due to hard seed coat.

Acknowledgements

The author would like to acknowledge Mahendra Ratna Multiple Campus, Ilam, and the Prime Minister Agriculture Modernization Project (PMAMP) for providing the funds and requiring backup, support, and services during the conduct of this research. The author would like to express her immense gratitude to an advisory committee for technical support and guidance Countless thanks to farmers in Godak, 10-Ilam, during research fieldwork.

References

Adelani, D. O., and M. I. Bello, 2016. Effect of fresh cow milk and coconut milk on the germination of *Tamarindus indica* seeds. *Applied Tropical Agriculture,* 21(3), 37-45.

Karthikeyan, C., D. Veeraragavathatham, D. Karpagam, and S. Firdouse, 2006. Cow based indigenous technologies in dry farming. *Indian journal of Traditional Knowledge,* 5(1), 47-50.

Khan, M., A. Ayaz, G. Ayub, and S. A. A. Shah, 2019. Influence of gibberellic acid concentrations and dipping durations on growth and yield of bitter gourd. *Sarhad Journal of Agriculture*, *35*(2), 587-593
DOI: 10.17582/journal.sja/2019/35.2.587.593

Koirala, N., D. Poudel, G. Bohara, N. Ghimire, and A. Devkota, 2019. Effect of seed priming on germination emergence and seedling growth of spring rice (*Oryza Sativa* L)cv.Hardinath-1. *Azarian Journal of Agriculture*, (6),7-12
DOI: 10.29252/azarinj.002

Kumhar, R. N., B. Baheti, and B. Chandrawat, 2018. Treatment of maize seed with cow-products: An eco-friendly management of maize cyst nematode (Heteroderazeae). *International Journal of Agriculture Science*, 10(9), 969-5971.

Malika, S., and S. Rawat, 2020. Effects of seed priming and it's duration on bitter. *Journal of Pharmacognosy and Phytochemistry*, 9(6),1950-1953.
DOI: 10.22271/phyto.2020.v9.i6ab.13237

Morshed, M., S. Rahman, I. Ahmed, S. Biswas, P. C. Sarker, A. Hossain, M. Islam, T. Jahan, and A. K. Choudhury, 2021. Effect of Milk and Hydropriming on Seed Germination Characteristics of Bitter Gourd (Momordica charantia L.). *Turkish Journal of Agriculture - Food Science and Technology*, *9*(2), 296-300.
DOI: 10.24925/turjaf.v9i2.296-300.3856

Palada, M. C., and L. C. Chang, 2003. Suggested cultural practices for bitter gourd. *Shanhua, Tainan: AVRDC - The World Vegetable Center* 03-547

Saleem , M. S., M. Sajid, Z. Ahmed, S. Ahmed, N. Ahmed, and M. Shahid, 2014. Effect of Seed Soaking On Seed Germination and Growth of Bitter Gourd Cultivars. *Journal of Agriculture and Veterinary Science (IOSR-JAVS)* ,6(6), 07-11
DOI: 10.9790/2380-0660711

Shinde, V. V., and K. V. Malshe, 2015. Effect of cattle urine and cowdung slurry as seed treatment on Khrini (Manikara hexandra L.). *Journal of ecofriendly agriculture* ,10(2) 128-130

Sowmya, H. M., S. Kolakar, S. Nadukeri, D. Lakshmama, V. Srinivasa, and S. Jakkeral, 2018. Influence of seed priming with gibberlic acid on antidiabatic vine - bitter gourd (*Momordica charantia* L.) Genotypes. *Journal of Pharmacognosy and Phytochemistry,* (3), 161-164.

Sridhar, S., and S. A. Kumar, 2013. Seed treatment techniques. *Centre for Indian Knowledge Systems (CIKS) Seed Node of the Revitalising Rainfed Agriculture Network.*

Tania, S., M. Hossain, and A. Hossain, 2019. Effects of hydropriming on seed germination, seedling growth and yield of. *Journal of Bangladesh Agricultural University*, 17(3), 281–287
DOI: 10.3329/jbau.v17i3.43197

Tiwari, S., A. K. Chaurasia, N. Nithyananda, and B. Bara, 2018. Effect of organic priming on seed germination behaviour and vigour of chickpea (*Cicer arietinum* L.). *Journal of Pharmacognosy and Phytochemistry,* 7(4): 1064-1067

A Comparative Study of the Seismic Behavior of Composite (Steel-Concrete) and Reinforced Concrete Structure

Rajan Suwal[1*], Pasang Chhiring Sherpa[1], and Nitesh Bhandari[1]

[1]Department of Civil Engineering, Pulchowk Campus, IOE, TU, Nepal

***CORRESPONDING AUTHOR:**

Rajan Suwal

Email:rajan_suwal@ioe.edu.np

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI :

https://doi.org/10.3126/njst.v21i2.67166

Date of Submission: May 14, 2023

Date of Acceptance: May 7, 2024

ABSTRACT

The low weight, high strength, and durability of steel-concrete composite construction have made it quite popular worldwide. However, it is not commonly used in Nepal. For the country, avoiding composite construction when it is economically viable is a great loss. Using composite materials is of particular interest due to their potential to improve performance through rather small changes in manufacturing and construction technologies. Steel concrete composite construction means the concrete slab is connected to the composite beam with the help of shear connectors so that they act as a single unit. In this paper we intend to compare the parameters like storey drift, time period, deflections, etc. between composite and RC structures for 6, 10 and 15 stories regular and irregular buildings situated in earthquake zone V. ETABS software was used for the analysis and linear static method, linear time history method and static pushover method were performed. The results are compared and we found out that composite constructions are more suitable in multi- storied buildings compared to RC structures.

Keywords: Composite buildings, Storey drift, Shear connectors, Seismic performance, Concrete-Steel Section.

1. Introduction

In Nepal, concrete is the most used, convenient and economical construction material in low-rise buildings. Nowadays due to overcrowding of city areas, the need for high-rise buildings has arisen. For medium to high-rise buildings RC structure is not suitable because of the increased dead load, requirement of formwork (which makes the construction process longer) and increase in cost. So, for these structures, steel-concrete composite construction can provide better performance and an effective economical solution**.** The main benefit of composite elements is that the properties of each material can be combined to form a single unit that performs better overall than its separate constituent parts.

Panchal & Marathe (2011) worked on steel-concrete composite, steel, and RC structure options which are considered for the comparative

study of G+30 storey commercial building which is situated in earthquake zone IV. The equivalent Static Method of Analysis is used. The comparative study includes deflections of the members, size and material consumption of members in composite concerning RC and Steel sections, seismic forces and behaviour of the building under the seismic condition in composite with respect to RC and Steel. He concluded that composite structure was more economical.

Prajapati & Panchal (2013) discussed the analysis & design procedure adopted for the evaluation of symmetric high-rise multi-storey buildings (G+30) under the effect of Wind and Earthquake forces. In these building, RC, Steel & Composite buildings with shear walls were considered to resist lateral forces resisting system. This study examines G+30 stories building that are analyzed and design under the effect of wind and earthquake using ETABS. A total of 21 numbers of various models are analyzed by equivalent static method and it proves that steel-concrete composite building is the better option.

Mahajan & Kalurkar (2016) performed a performance analysis of RC and steel concrete composite structures under seismic effect. They showed the effect of FEC (Fully Encased Composite) on a G+ 20-storey special moment frame. The linear static analysis and nonlinear static analysis i.e. "Pushover analysis" is done for G+20-storey structure. Results are compared for the base shear, modal time period, storey displacement and storey drift for both structures. As the composite has more lateral stiffness, the results of the time period and storey displacement show significant variation. While analyzing for "Non-linear static analysis the performance point for the FEC is significantly much more as compared to the RC model.

Shah & Pajgade (2013) performed a comparative study of RC with a Composite (G+15) Storey building. Steel-concrete composite with RC options is considered for the comparative study of G+15 storey office building which is situated in earthquake zone IV & wind speed 39 m/s. An Equivalent Static Method of Analysis was used. For the modeling of Composite & RC structures, STAAD-Pro software was used. The results were compared and it was found that composite structures are more economical.

Etli & Güneyisi (2020) performed a seismic performance evaluation of regular and irregular composite moment-resisting frames. The seismic behavior of regular and irregular composite moment-resisting frame buildings was investigated. 5, 8, 10, 13 and 15-story composite moment-resisting frames having concrete-filled steel tube columns and composite beams were designed at high ductility levels and their performances were evaluated comparatively.

Uddin & Azeem (2020) performed a comparative study on the seismic behaviour of composite and RC plan irregular structures. All the models considered are G+15 storey and are irregular in plan and the irregularity condition as per IS 1893-2002 is satisfied resulting in a T shape and a Plus Shape models.

Anagha S. S & Raghu K (2023) performed a comparative study on the seismic behaviour of RC and Composite structures for different types of irregularities. ETABS is used to model and analyze the comparison of RC and composite structures with CFT columns for different kinds of irregularities, such as vertical geometric irregularity, mass irregularity, and stiffness irregularity, in accordance with IS codes.

Pannirselvam & Sreelekshmi (2022) performed a study on irregular tall RC structures and composite structures by pushover analysis. They addressed the study and behaviour of structures with composite columns–concrete-filled steel tube columns (CFST) having irregularities in plan and elevation, subjected to ground motion. In this paper, a study on how composite column–concrete-filled steel column meets seismic demands in five irregular structures and its advantages over RC has been carried out by pushover analysis in ETABS.

After reviewing all these works, we set the following as the objective of our study:

- To investigate the seismic behavior of steel-concrete composite frames over the reinforced concrete (RC) frames in both regular and irregular buildings.
- To compare ductility by performing inelastic (pushover) analysis of steel-concrete composite sections and RC sections.

2. Materials and Methods

2.1 Building details

3D modeling of all the RC and composite buildings was done using ETABS software. The models used are not the real existing buildings. 12 models were used in this research: G+5, G+9, G+14 regular composite buildings, G+5, G+9, G+14 regular RC buildings, G+5, G+9, G+14 irregular composite buildings and G+5, G+9, G+14 irregular RC buildings. The plan of the irregular building is chosen to consider torsional irregularity i.e. 1st or 2nd mode of the building istorsion. A floor plan of 28m x 28m dimensions was considered for regular structures in this study where the center-to-center distance between two grids is 4 m as shown in Fig.s 2.1 to 2.4.

The sizes of the beam, column and slab used are illustrated in Table 2.1. Different sections are used in different storey buildings as per design criteria given in IS 456: 20000 and AISC 360-16.

The loading conditions used in the analysis and all other parameters considered in the design according to IS 875(1987-Part 1), IS 875(1987-Part 2), IS 1893, Part 1 and IS: 800:2007, are provided in Table 2.2.

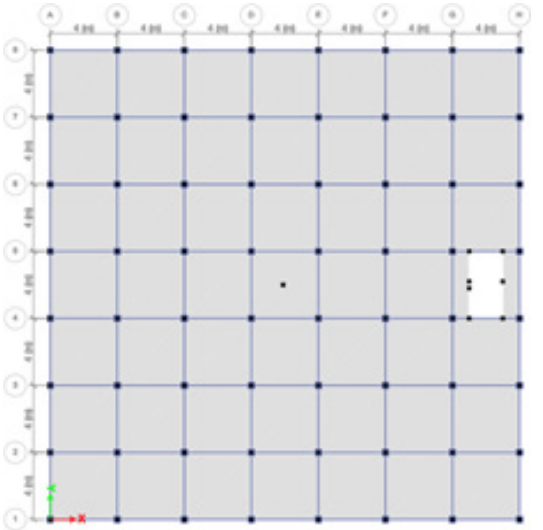


Fig. 2.1: Plan of regular buildings

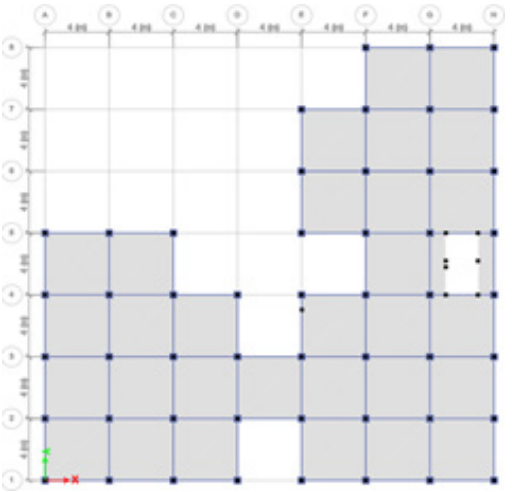


Fig. 2.2: Plan of irregular buildings

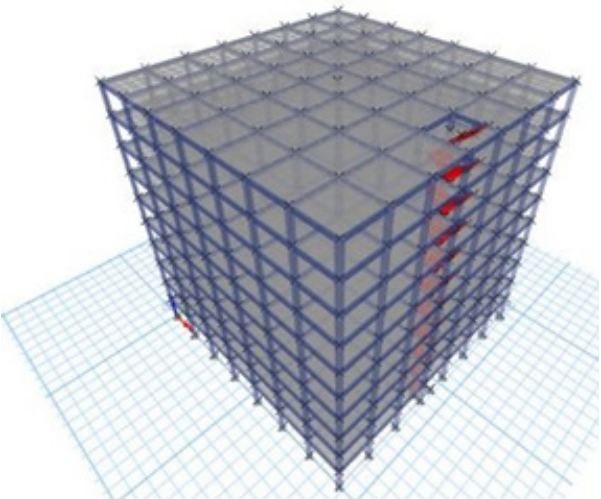


Fig. 2.3: 3D view for regular RC composite structures

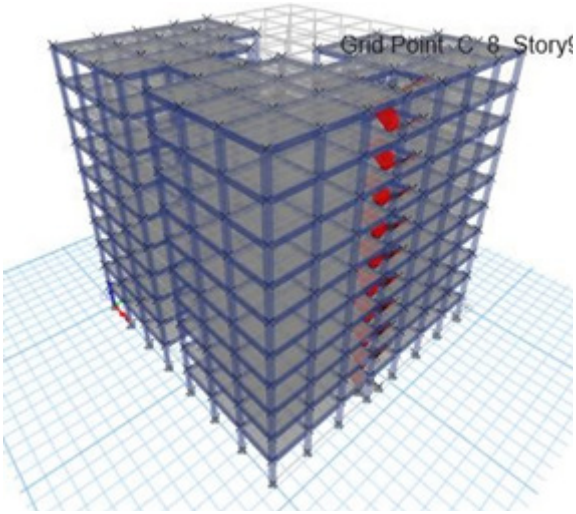


Fig. 2.4: 3D view for irregular RC and composite structures

Table 2.1: Column and beam sizes of structure

Building type	Plan	Story	Column size(mm*mm)	Beam size (mm*mm)	Secondary beam	Slab and deck size
RC	Regular	6	375*375	350*275	-	200
		10	400*400	350*275	-	200
		15	450*450	450*350	-	200
	Irregular	6	375*375	350*275	-	200
		10	450*450	375*300	-	200
		15	500*500	500*400	-	200
Composite	Regular	6	400*400 (ISHB-200)	ISMB-225	ISJB-175	200
		10	400*400 (ISHB-200)	ISMB-225	ISJB-200	200
		15	450*450 (ISHB-225)	ISMB-300	ISJB-175	200
	Irregular	6	400*400 (ISHB-200)	ISMB-225	ISJB-175	200
		10	400*400 (ISHB-200)	ISMB-225	ISJB-200	200
		15	450*450 (ISHB-225)	ISMB-300	ISJB-175	200

Table 2.2: Basic loadings and parameters considered for the design

Seismic zone	V
Soil condition	Soft soil
Floor finish	1.5 kN/m^2
Live load at all floors	4.0 kN/m^2
Live load at staircase	4.0 kN/m^2
Zone factor	0.36
Importance factor	1.0
Grade of concrete	M30
Grade of structural steel	Fe345
Grade of rebar	HYSD500
Response reduction factor	5 for SMRF

2.2 Analysis

Structural analysis of all the RC and composite buildings was done using ETABS software. The analysis procedure used in ETABS was the linear static method, linear time history method and static pushover method. Here the conventional RC structure is designed according to IS 456-2000 and the composite structures is designed according to the AISC 360-16 code provisions.

2.2.1 Linear Static Procedure

A linear static analysis is an analysis where a linear relation holds between applied forces and displacements. The linear static procedure is a method of estimating the response of the structure to earthquake-induced forces by representing the effects of this response through the application of a series of static lateral forces applied to an elastic mathematical model of the structure and its stiffness. The forces are applied to the structure in a pattern that represents the typical distribution of inertial forces in a regular structure responding linearly to the ground shaking excitation, factored into account (here response reduction factor of 5 is as per IS code for both composite and RC structure), in an approximate manner, for the probable inelastic behavior of the structure. In a linear static analysis, the model's stiffness matrix is constant, and the solving process is relatively short compared to a nonlinear analysis on the same model. Therefore, in this paper, for a first estimate, the linear static analysis is used prior to performing a full nonlinear analysis.

2.2.2 Linear Dynamic Procedure

The main purpose of linear dynamic analysis is to \] evaluate the time variation of stresses and deformations in structures caused by arbitrary dynamic loads. In this paper linear time history analysis as per the IS code 1893:2016 is performed for linear dynamic procedure. As per ASCE 7-10(2010), a minimum of seven ground motion histories should be considered in the linear dynamic procedure. So, seven ground motion acceleration histories having magnitudes, fault distances, and source mechanisms consistent with seismic hazard at the design location were selected

from the PEER NGA [14] database. The two horizontal ground motion components of each pair were scaled to match with target spectrum, and response spectrum given for the soft soil as per IS code 1893:2016 and were applied in orthogonal directions along the principal axes of the building structure.

Table 2.3: Criteria for ground motion selection

Magnitude (min, max)	6.5-8 Mw
Fault type	Reverse + Oblique
Fault distance	20-100km
Seismic shear wave velocity (Vs30)	180-360 m/s (soft soil)

Table 2.4: Ground motions

Earthquake Name	Year	Station Name	Magnitude (Mw)	Mechanism	Rjb (km)	Rrup (km)	Vs30 (m/sec)
"San Fernando"	1971	"2516 Via Tejon PV"	6.61	Reverse	55.2	55.2	280.56
"San Fernando"	1971	"Carbon Canyon Dam"	6.61	Reverse	61.79	61.79	235
"Friuli_Italy-01"	1976	"Codroipo"	6.5	Reverse	33.32	33.4	249.28
"Friuli_Italy-01"	1976	"Conegliano"	6.5	Reverse	80.37	80.41	352.05
"Tabas_ Iran"	1978	"Boshrooyeh"	7.35	Reverse	24.07	28.79	324.57
"Tabas_ Iran"	1978	"Ferdows"	7.35	Reverse	89.76	91.14	302.64
"Taiwan SMART1(25)"	1983	"SMART1 C00"	6.5	Reverse	95.57	96.06	309.41

2.2.3 Non-Linear Static Procedure

In reality, during earthquakes, buildings are generally subjected to large inertia forces which cause members of buildings to behave in a nonlinear manner. Thus, the earthquake shaking of the structure is a nonlinear dynamic problem and structural analysis should incorporate the nonlinear behavior of members for evaluating the actual response of the structure. Non-linear static analysis (also known as pushover analysis) is a procedure where a mathematical model incorporating the inelastic post-yield behavior of the structural elements is subjected to monotonically increasing horizontal loads until target displacement is reached. It is generally used to evaluate the performance point and the weak link of structures. In this paper, the performance point is calculated using the capacity spectrum method, where both the capacity curve and the demand curve are converted into acceleration displacement response spectrum (ADRS) format i.e. spectral acceleration vs spectral displacement. The intersection point of the converted demand and capacity curve is the performance point.

3. Results And Discussions

The following results were observed from the analysis and comparison of results between RC and Composite structures is shown through the graph:

3.1 Regular Buildings

3.1.1. Story Displacement

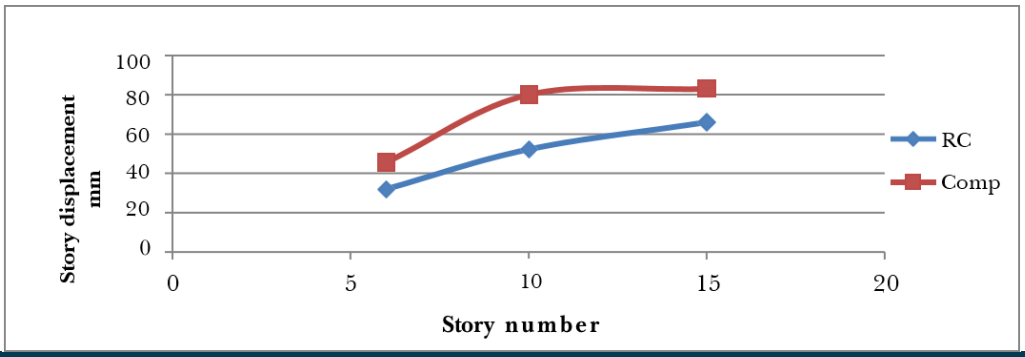


Fig. 3.1: Comparison of displacement X

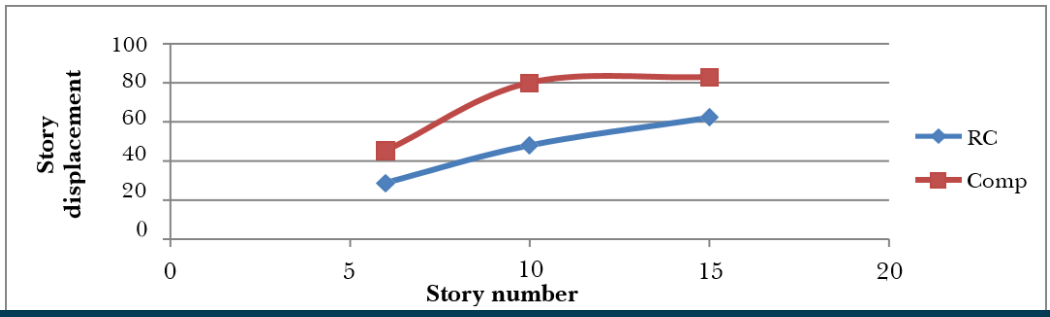


Fig. 3.2: Comparison of displacement Y

3.1.2. Story drift ratio

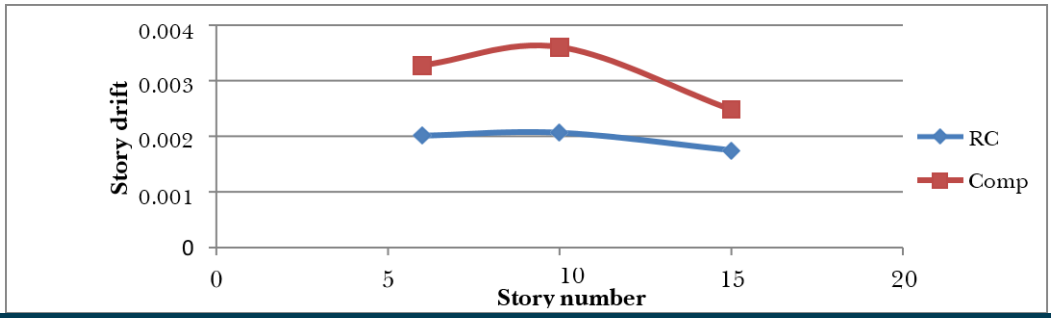


Fig. 3.3: Comparison of story drift X

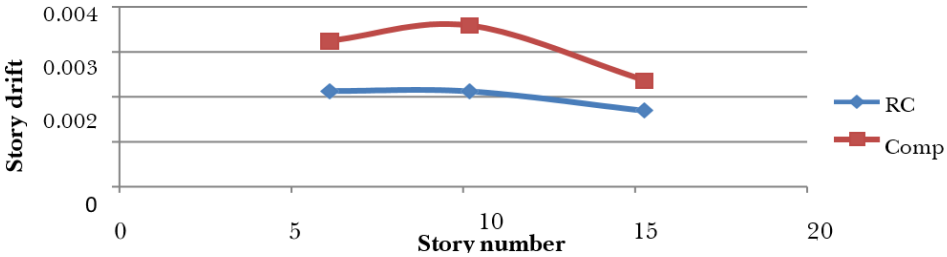


Fig. 3.4: Comparison of story drift Y

3.1.3. Overturning moment

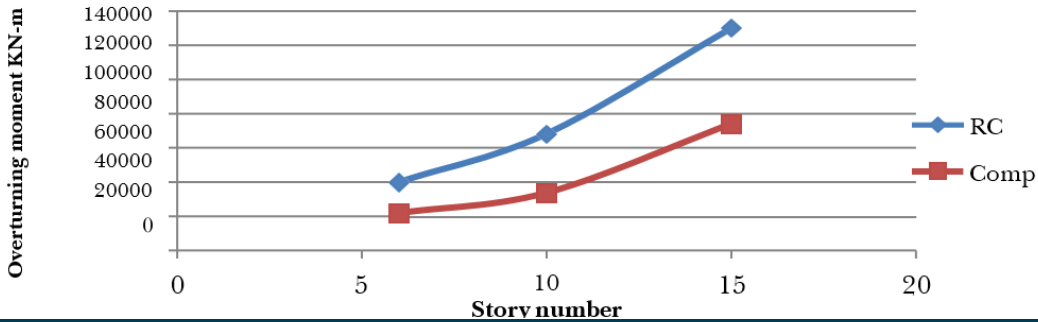


Fig. 3.5: Comparison of overturning moment X

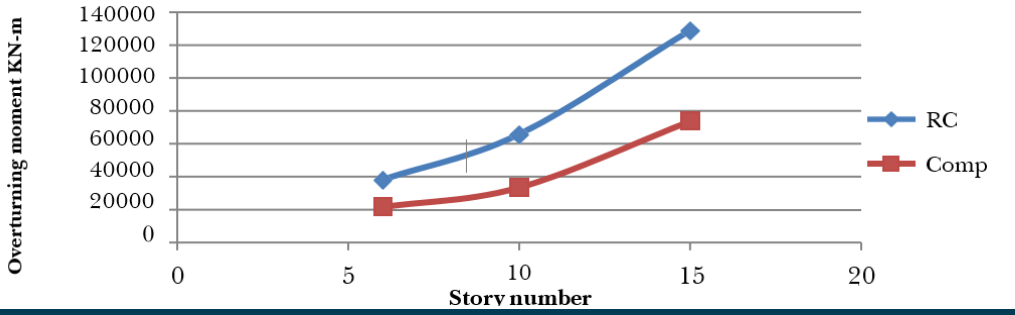


Fig. 3.6: Comparison of overturning moment Y

3.1.4. Time period

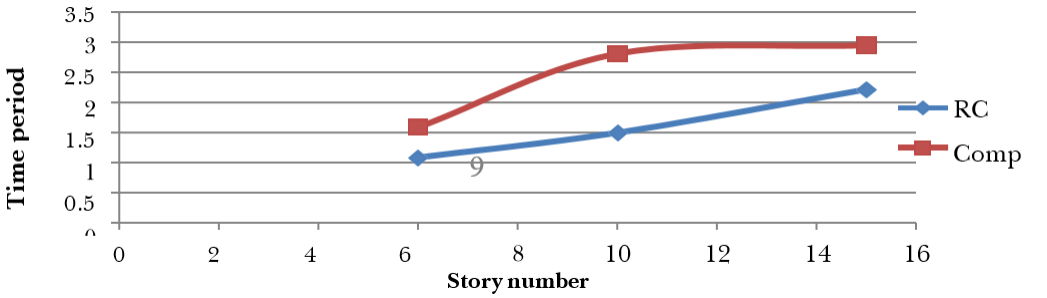


Fig. 3.7: Comparison of time period X

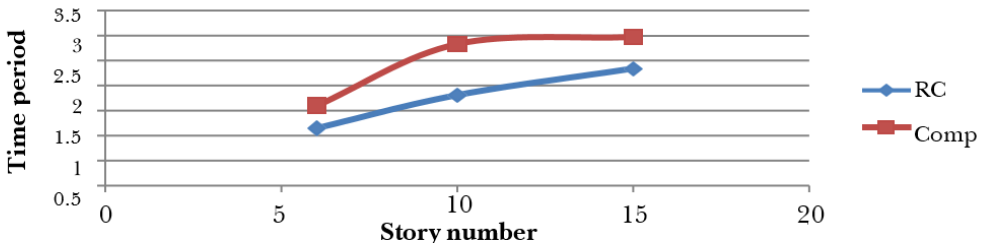


Fig. 3.8: Comparison of time period Y

3.1.5. Base shear

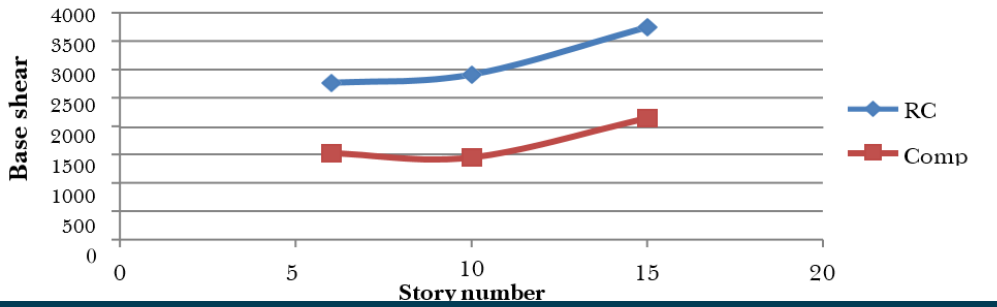


Fig. 3.9: Comparison of base shear X

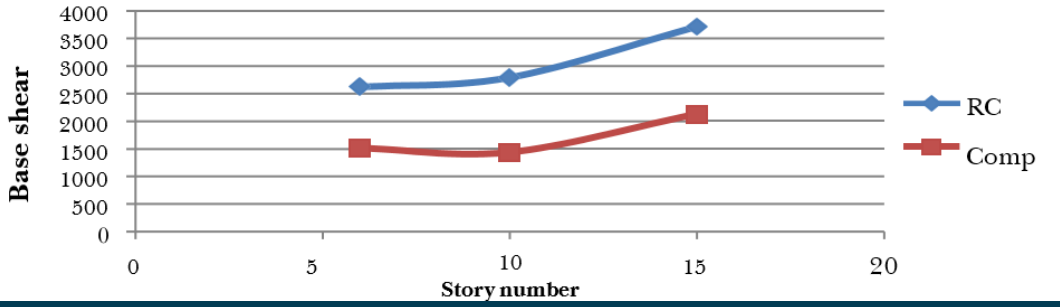


Fig. 3.10: Comparison of base shear Y

3.1.6. Performance point (spectral displacement)

Story Number	Direction	RC (mm)	Composite(mm)	Percentage Change
6	X	136.97	210.82	53.92
	Y	131.48	208.86	58.85
10	X	268.49	312.18	16.27
	Y	226.04	308.32	36.40
15	X	297.44	384.61	29.30
	Y	289.54	381.66	31.77

3.2 Irregular Buldings

3.2.1 Story displacement

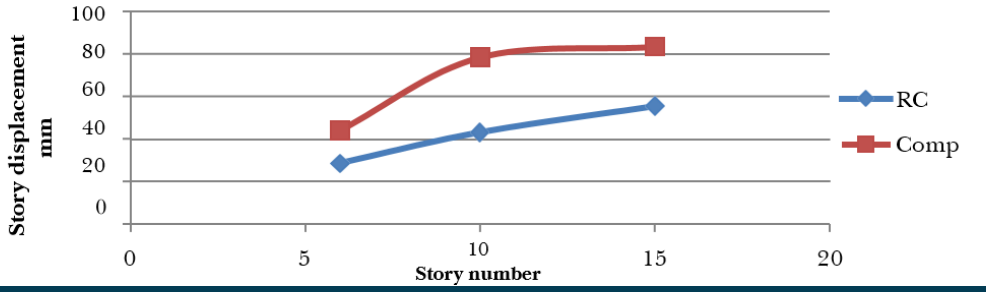


Fig. 3.11: Comparison of story displacement X

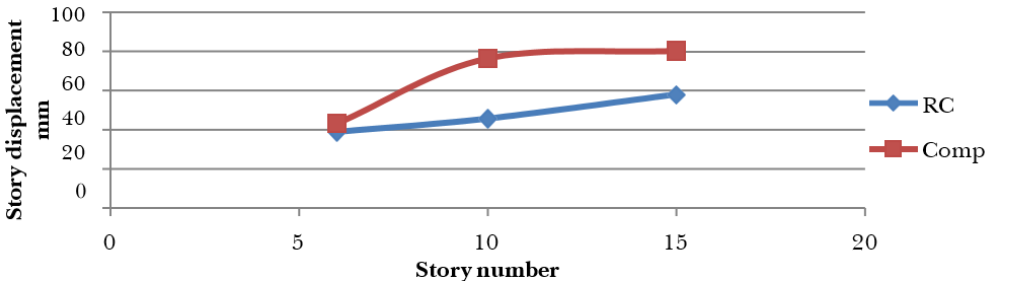


Fig. 3.12: Comparison of story displacement Y

3.2.2. *Story drift*

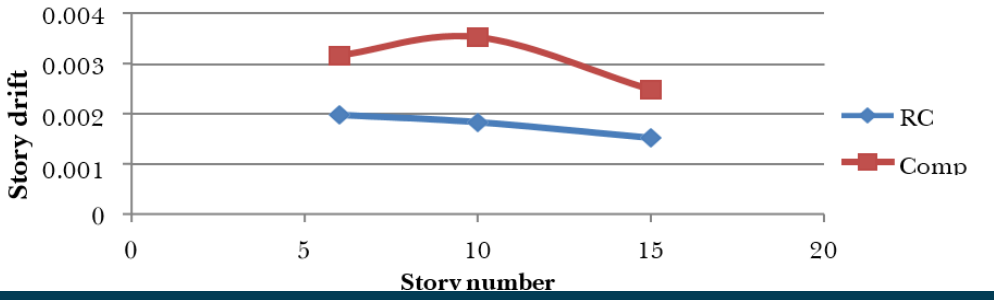


Fig. 3.13: Comparison of story drift X

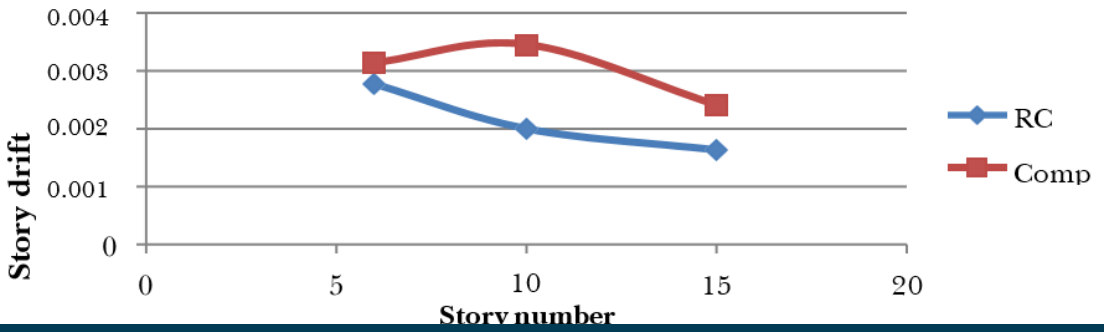


Fig. 3.14: Comparison of story drift Y

3.2.3. *Overturning Moment*

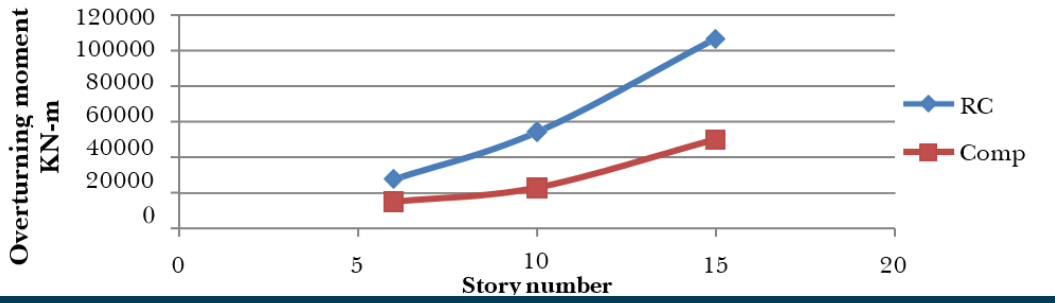


Fig. 3.15: Comparison of overturning moment X

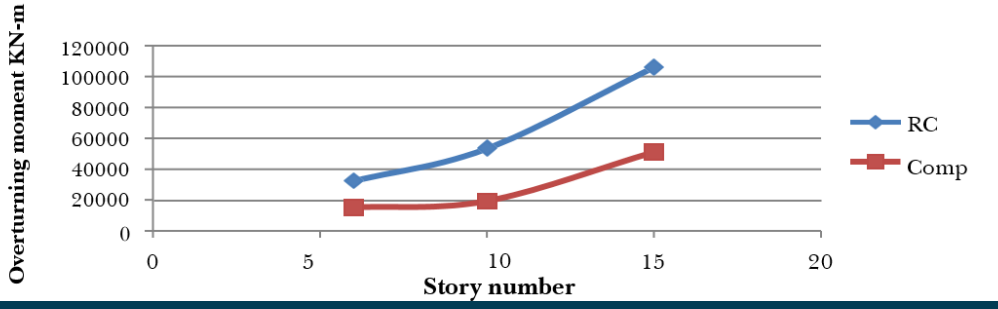


Fig. 3.16 : Comparison of overturning moment Y

3.2.4. Time Period

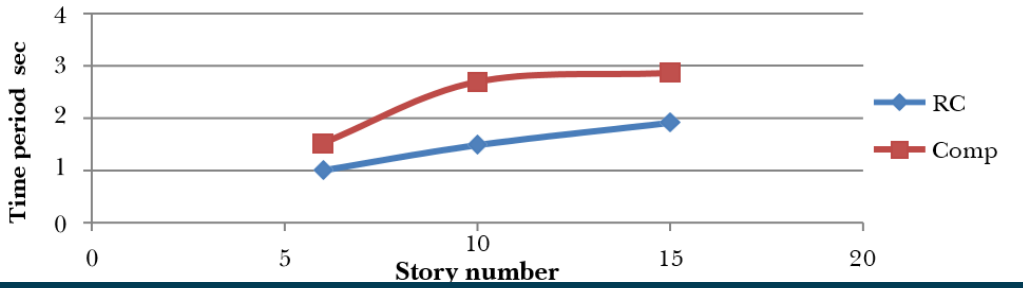


Fig. 3.17 : Comparison of time period X

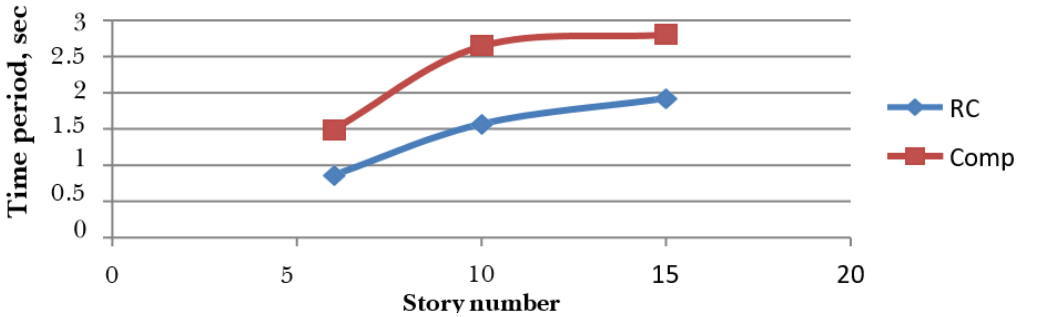


Fig. 3.18 : Comparison of time period Y

3.2.5. Base Shear

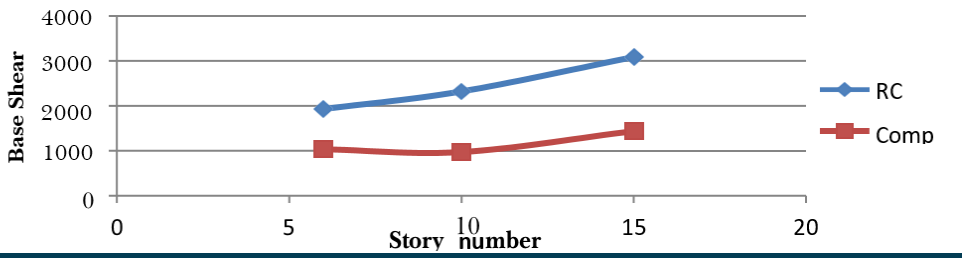


Fig. 3.19 : Comparison of base shear X

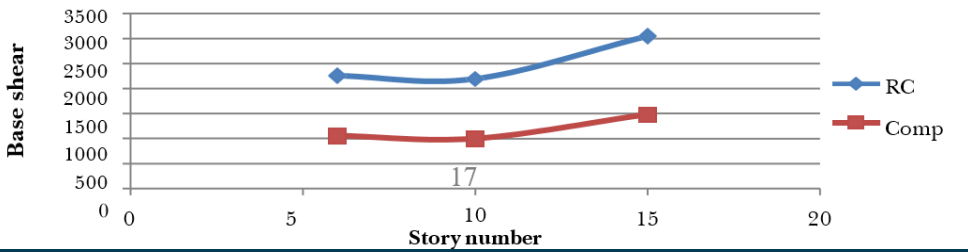


Fig. 3.20 : Comparison of base shear Y

3.2.6. Performance point (spectral displacement)

Table 3.5: Performance point (spectral displacement)

Story Number	Direction	RC (mm)	Composite(mm)	Percentage Change
6	X	161.84	207.12	27.98
	Y	134.82	203.18	50.70
10	X	210.67	302.24	43.46
	Y	209.59	293.94	40.25
15	X	293.55	374.02	27.41
	Y	292.54	363.85	24.38

Shear force of column (C1)

Table 3.6: Shear force of column (C1)

Story number	RC (kN)	Composite(kN)
6	36.12	19.20
10	39.44	20.84
15	55.24	29.37

Bending Moment of column (C1)

Table 3.7: Bending Moment of column(C1)

Story number	RC (kN-m)	Composite(kN-m)
6	93.25	78.79
10	101.16	80.42
15	131.19	98.33

Axial Force of column (C1)

Table 3.8: Axial Force of column(C1)

Story number	RC (kN)	Composite(kN)
6	174.30	141.27
10	823.94	549.92
15	1799.84	1042.00

4. Discussion

Composite buildings have a high time period, high maximum story displacement, and high story drift ratio compared to RC buildings, making them more flexible to oscillate in response to lateral forces. Axial force, maximum bending moment & shear force in a column of the RC structure are on the higher side than that of the composite structure for both regular and irregular configuration. Also, the higher performance point in composite buildings than RC buildings indicates higher ductility of composite structures as compared to RC which is best suited to the effect of lateral forces. In composite buildings, seismic forces are less than in RC structures, which may be attributed to their lighter seismic weight.

5. Conclusion

- For regular configuration the lateral displacements of composite structure are found to be 26% to 53% more in the Y-direction and about 32% to 66% more in X direction than the RC structures and for irregular configuration, the lateral displacements of composite structure are found to be 11% to 67% more in Y-direction and about 50% to 82% more in X direction than the RC structures. In irregular configuration lateral displacement is higher than regular.
- For regular configuration the maximum story drift ratio of the composite structure is found to be 22% to 59% more in the Y-direction and about 42% to 74% more in X direction than the RC structures and for irregular configuration the maximum story drift ratio of the composite structure is found to be 12% to 72% more in Y-direction and about 50% to 82% more in X direction than the RC structures.
- For regular configuration the overturning moment of the composite structure is found to be 42% to 48% less in the Y-direction and about 42% to 50% less in X direction than the RC structures and for irregular configuration, the overturning moment of the composite structure is found to be 51% to 54% less in the Y-direction and about 46% to 57% less in X direction than the RC structures.

- For regular configuration the time period of the composite structure is found to be 26% to 56% more in the Y-direction and about 33% to 62% more in X direction than the RC structures and for irregular configuration the time period of the composite structure is found to be 45% to 73% more in Y-direction and about 49% to 81% more in X direction than the RC structures.
- For regular configuration the base shear of the composite structure is found to be 42% to 48% less in the Y-direction and about 42% to 50% less in X direction than the RC structures and for irregular configuration, the base shear of the composite structure is found to be 51% to 54% less in Y-direction and about 46% to 57% less in X direction than the RC structures.
- From pushover analysis, for regular configuration the performance point of the composite structure is found to be 31% to 58% more in the Y-direction and about 16% to 53% more in X direction than the RC structures and for irregular configuration, the time period of the composite structure is found to be 24% to 50% more in Y-direction and about 27% to 43% more in X direction than the RC structures.

All the percentage variation in different parameters between RC and composite sections shows us that composite sections are efficient in high-rise buildings. Also, the sections used in composite sections are on the lower side which maximizes the space and lowers the dead load. The weight of composite structure is quite low as compared to RC structure which helps in reducing the foundation cost. An emphasis on speedy construction facilitates a faster return on investment & a greater rent-related benefit. That is why we can say that composite buildings are more economical than RC buildings.

References

Mahajan, A. S., L. G. Kalurkar, 2016. "Performance analysis of RCC and steel concrete composite structure under seismic effect", International Journal of Research in Engineering and Technology eISSN: 2319-1163 | pISSN: 2321-7308, Volume: 05 Issue: 04.
DOI: 10.15623/ijret.2016.0504015

AISC 360-16, 2016. An American National Standard Specification for Structural Steel Buildings.

Anagha S. S., K. Raghu, 2023. "Comparative Study on Seismic Behaviour of RC and Composite Structure for Different Types of Irregularities", Journal of Mines, Metals & Fuels, Vol 71, Issue 7, p964.

Shah, A. N., P. S. Pajgade, 2013. "Comparison of RCC and Composite Multistoried Buildings", International Journal of Engineering Research and Applications (IJERA), ISSN: 2248-9622, Vol.3, Issue 2, pp.534-539.

Prajapati, B. D. and, D. R. Panchal, 2013. "Comparative Study of R.C.C, Steel and Composite (G+30 Story) Building", International Journal of Advances in Engineering & Technology, ISSN: 22311963, Vol. 6, Issue 4, pp. 1836-1847.

Panchal, D. R., P. M. Marathe, 2011. "Comparative Study of R.C.C, Steel and Composite (G+30 Story) Building", Institute of Technology, Nirma University, Ahmedabad-382481.

IS 456:2000, "Indian Standard Code of Practice for Plain and Reinforced Concrete", Bureau of Indian Standards, New Delhi, India.

IS 875(1987-Part 1), Code of Practice for Design Loads (Other than Earthquake) for Buildings and Structures, Dead loads, Bureau of Indian Standards (BIS), New Delhi.

IS 875(1987-Part2), Code of Practice for Live Loads, Bureau of Indian Standards (BIS), New Delhi, India.

IS: 1893, Part 1, 2002. "Criteria for Earthquake Resistant Design of Structures - General Provisions for Buildings", Bureau of Indian Standards, New Delhi.

IS: 800:2007, "General Construction in Steel - Code of Practice", Bureau of Indian Standards, New Delhi, 2007.

Uddin, M. A., M. A. Azeem, 2020. "Comparative Study on Seismic Behaviour of Composite and RCC plan Irregular Structures", International Journal of Engineering Research & Technology (IJERT) ISSN: 2278-0181, Vol. 9 Issue 01.
DOI: 10.17577/IJERTV9IS010099

Narayanan, P., M. Sreelekshmi, 2022. Study on Irregular Tall RC Structure and Composite Structure by Pushover Analysis. In: Loon, L.Y., Subramaniyan, M., Gunasekaran, K. (eds) Advances in Construction Management. Lecture Notes in Civil Engineering, vol 191.

PEER, 2014. Pacific Earthquake Engineering Research Center (PEER). Pacific Earthquake Engineering Research Center.

Etli, S., E. M. Güneyisi, 2020. "Seismic performance evaluation of regular and irregular composite moment resisting frames", Latin American Journal of Solids and Structures, 17(07).
DOI: 10.1590/1679-78255969

Science Communication and Role of Media in Nepal

Raju Adhikari[1*], Suresh Kumar Dhungel[2] and Bharat R Poudel[1]

[1]Nepal Science Foundation Trust, Skill Knowledge and Technology Transfer Department, NRNA Kathmandu, Nepal.
[2] Nepal Academy of Science and Technology, Nepal.

***CORRESPONDING AUTHOR:**
Raju Adhikari
Email:adhikari2428@gmail.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI :
https://doi.org/10.3126/njst.v21i2.67167

Date of Submission: Mar 16, 2023

Date of Acceptance: Apr 16, 2023

ABSTRACT

The importance of communicating scientific knowledge was realized very early in the developed countries. Today most universities and educational institutions have devised science and journalism courses and use innovative approaches to increase the understanding and application of science knowledge for access to a general audience. The exponential growth of information technology and growing interests >90% seeking science related information in social, news and print media has helped science communication flourish in developed countries. On the other hand, in the developing countries science communication is still not a priority. Of various S&T institutions, National Academy of Science and Technology (NAST) stands out for taking major initiative. The advancement of communication technology and changing dynamics of social media due to internet, facebook and google access has helped Nepal to raise social awareness but providing evidenced based information remains a low priority. The chapter attempts to review the history of science education and communication in Nepal and the role of Nepalese media and global media trends. The information presented is based on published and unpublished literature and feedback from S&T institutions and schools, policymakers, school teachers, students, parents and community leaders. A list of Key performance indicators (KPIs) such as funding source, teachers' availability, students' enrolments, resource availability and development trends of the science education and community science awareness program in regional and remote areas are discussed.

Keyword: Science, Communication, Science education, Media, Information

1. Introduction-Science Communication

Science communication is the practice of informing, educating, raising awareness of evidenced based information for greater benefit of the people and planet. Science communication is classed in two different types i) outreach (Scientists to non-expert audiences) and ii) in reach (communication with scientific backgrounds) (Fig. 1).

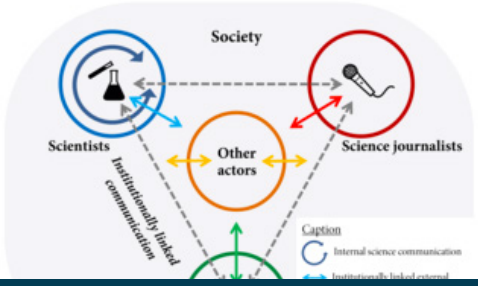


Fig. 1 : Schematic overview of the field and the actors of science communication according to Carsten Könneker (https://en.wikipedia.org/wiki/Science_communication)

Science communication has a long and deep-rooted history and was driven by scientific discovery before it has reached this stage of digital communication age). Science communication is essentially described in three stages of development. The first generation of science communication centred on filling in the gaps in the knowledge of the general public. The second-generation approach favoured a more two-way dialogue, and the third-generation approach aims to continue two-way dialogue, but also transfers greater ownership to the general public.

In ancient Greece, imparting knowledge was shared in public debate by the masses. Such debates triggered logical analysis and experimentation which later helped in the advancement of philosophy and science. Later scientific discoveries from knowledge generated from such debates were translated in writing but unfortunately the masses were unable to process the knowledge described in writing. With the growing literacy and the invention of the printing press in 1456 eventually helped spread the knowledge to broader masses, increased literacy and the responsibility to communicate knowledge to the general public (Roberts-Artal 2015).

In the 19 centuries, British Science Association (BSA) first meeting held in York in September 1831, brought national attention to science discovery and communication and inspired the formation of several professional associations for the advancement of science globally, and created a forum for an annual meeting.

The late 20th century could be called a golden start of science communication "and" public engagement. Michael Faraday a famous scientist who discovered electricity, used to run lectures aimed at the non-expert public which began in 1825. The 20th century saw science positioned in a broader cultural context and allowed scientists to communicate their knowledge in a way that could reach and be understood by the general public. Science can be communicated to the public in many different ways. Traditional journalism is one way communication. The second category is live or face-to-face events through public lectures, debates, science busking "sci-art" exhibits, science cafés and science festivals, citizen science or crowd-sourced science, is a two-way science communication. The third category is online interaction; for example, websites, blogs, wikis and podcasts and other social media.

With the advent of citizen science, the general public are now in a position where they are not only choosing what they want to be informed about, but are taking an active role in the pursuit of this knowledge. Citizen Science is a phrase that is currently dominant in scientific communication circles. It is a forum of collaborative research with the general public (or citizens), which helps in collecting and generating data Human Genome Project, the world's largest collaborative biological project is a great example of science for and on behalf of the greater good (Roberts-Artal 2014).

Several countries have now adopted the policy to encourage research organizations to communicate about their research activities and results widely and to the general public. All research projects now have an obligation to integrate a communication plan to increase the public visibility of the project using an accessible language and adapted channels and materials. It is a globally accepted fact that the status of science communication and popularization of any country is one of the indicators of its economic prosperity.

2. Science Communication in Nepal

In Nepal, the political change in 1950 is being marked as the beginning of science and technology activities which led to establishment of a number of S&T related institutions and infrastructure, promotion and dissemination of scientific information. In Nepal, where

only 20% of schools teach science courses, science education and communication are not a priority. Both public and private science institutions, as well as professional societies, are doing little to advocate science education and communication largely due to budgetary constraints. Despite the national pledge for making science education as the basis for economic development and the promise to invest about 0.5% of national GDP in science and technology, lack of international funding, scientific vision, approach and leadership have severely hampered the science education and communication in Nepal.

Despite different governments reiterating their commitment to S&T and science education in the last seven decades, there has never been a long-term commitment to strengthen existing institutions, infrastructures and skilled human resources. Nepal Academy of Science and Technology (NAST) has prepared a discussion paper on Scientific Research, Technology, Development, Transfer and Dissemination-Some Guiding Concepts in 1983 but it has not been materialized to the significant extent till date. In the following section, a bird's eye view of history of science education, science policy, institutions related to science and technology, national and international players active to promote S&T in Nepal, role of media for the promotion of science, and global media trend is discussed and is limited to natural science and technology. The author believes that it is important to understand the history of science education and media roles in perspectives to understand the current status of science communication in Nepal.

3. Brief History of Science Education in Nepal

Prior to modern education in Nepal, Sanskrit education based on Hindu religion scripture Vedas, Upanishada, Jyotish (Astronomy) and science of rituals (KarmaKanda) was the main source of knowledge and path to spiritualism known as eastern Philosophy. The Hindu religious scriptures and Vedas manifestation on ritual practices later gave rise to the traditional science and technology such as Ayurveda, Vedic mathematics and agriculture, Astrology, etc. Modern science education was integrated with the above knowledge and was considered as an inevitable tool to achieve a higher standard of living and a convenient life free from physical labor. In Nepal, the modern-day school education began from the year 1854 with the establishment of the first school called Durbar High School and after the major political shift in 1951, the Government put significant efforts to vitalize the education system. National education planning commission, national education committee and the national education advisory board were formed to reform the education system.

At the higher education level, Trichandra College established in 1919 was the only college to teach science and science was allowed as an extra subject for the school leaving certificate (SLC) examination. Tribhuvan University (TU) chartered in 1959, was the only institution to grant undergraduate to PhD degrees. TU colleges offer a wide range of courses in different disciplines such as social sciences, humanities, education, commerce and business, law, sanskrit, and natural sciences including medical sciences, engineering, agriculture, and forestry. The political change in 1990 which led to establishment of a multiparty democratic system of governance brought further changes in the education system of Nepal. Subsequent political change in 2007 manifested education as one of the fundamental rights of the people. The Government introduced a new national education policy in 2019 which laid emphasis on technical education and scientific methods in the education system, adoption of Science, Technology, Engineering, and Mathematics (STEM) education in Nepal. Despite importance to science education, it continues to be taught in isolation from social context and fails to capitalize the significance of science knowledge and communication to empower people and negate superstitions and pseudoscience.

Nepal National Educational Planning Commission (NNEPC) in 1961, recommended science as a compulsory subject from grade six to eight and optional subject in grades nine and ten with emphasis on management of science laboratories and effective teaching of science. Science was made a compulsory subject in all three types of schools viz., general, sanskrit and vocational secondary schools and emphasized on the construction of laboratories and teaching materials at the local level. This provision made establishment of science laboratories compulsory in every secondary school.

Sixth Five-year plan of Nepal (1980-1985) linked science and technology with economic development of Nepal and aimed at developing and promoting the S&T sector as the engine to drive the social, economic, physical, and environmental development. Over the

years, the education commission gave priority to science and one of the important milestones was the establishment of Science Education Project (SEP) in 1982, National Science Education Development Centre (SEDEC) and Science Education Development Units (SEDU) at 25 selected districts throughout the country. The National Council for Science and Technology initially established in 1960 for advisory policy making in S&T was later succeeded to the permanent function of Nepal Academy of Science and Technology (NAST, established in 1982 as the apex S&T body.

Nepal does not have a long history of written policy on S&T. The third and the latest policy on S&T was promulgated in 2019 by the Ministry of Education, Science and Technology as "National Science, Technology, and Innovation Policy, 2019". In Nepal, science policy is being revised in the interval of almost every 15 years but no national commitment was shown to implement the documents as such. Major objectives of the new policy are to ensure research-based quality education, use of innovative technology in the productive sectors, developing such environment for the scientific talents to carry out research, opportunity to exhibit inventions, ensuring enhancement of the entrepreneurship, restructuring of the existing research-based institutions, and creating conducive environment for scientific research and technology development by scaling up the investment and promoting collaboration and science communication. (Nepal Education since 1951; Bajracharya & Brouwer 2007; Gimeno 2019).

4. Role of Nepal Media and Science Communication

Even though the formal education of science began in Nepal with the Intermediate in Science (I.Sc.) program in Tri-Chandra College in 1919 and in School in 1939 as an extra subject, science subject quickly became popular as it created inquisitiveness in young minds and provided the opportunity for a professional career path. Science communication activities such as exhibitions, science fairs, and competitive quiz programs were conducted in a limited way by academic institutions. The history of science popularisation in real terms began through IDRC funded project by the Royal Nepal Academy of Science and Technology (RONAST) in 1984 (Huggan 1987). Earlier to this, Nepal media was not covering science features due to a lack of training and subject specialization in a contemporary science discipline. In the mainstream media, science simply did not find a dedicated space.

The above project was the first collaborative program on science communication with Nepalese media practitioners, publishers, and editors. The project focus was science popularization at the community level working with the press and radio of the country through local-language print, broadcast media, and with local newspapers. The Nepal Khabar was the to first publish science features-free of cost as a bi-monthly publication in the remote parts of the country and set a road map for other newspapers to publish science-related columns and articles. The Weekly Science and Technology Radio Program, aired on Radio Nepal, was another stepping success as 55-65% of the population had access to radio and benefited from the program. Nepal Radio Nepal's excellent network of relay stations provided a much-needed infrastructure.

The science program, delivered in a print format, covered general knowledge on science, the latest news from science labs around the world, interviews with various scientists, features on scientific institutions, and a radio quiz question-and-answer portion. More than 400 letters from 76 districts in Nepal were received by broadcasters working on the radio program and Radio Nepal science communication was hailed as the third most popular program. Mr. Gurung from the Centre of Education and Development Administration (CEDA) in his report on Science Popularization, highlighted the lack of funding and infrastructure to train science journalists as the most limiting factor The report recommendation led to the establishment of two specialized training workshops on science reporting and 40 science writers and broadcasters benefited from the workshops.

The strategies to work with editors and broadcasters on a continuing basis with coordinated efforts provided the much-needed foundation on the ground support. Radio Nepal and RONAST continued to work in the production of weekly science programs with stories of science and technology to everyday life through interviews. The establishment of science communication awards and prizes to encourage participation and ongoing review of the impact of the project's various components helped design a long-term science popularization program for Nepal. This strategy further helped 'establishment' of regular science columns or pages in the national newspapers, under science service produced by Rastriya Samachar Samiti (RSS Nepal's national news agency), and regular science and technology programs

broadcast by Radio Nepal (IDRC report 1992).

However, scientific learning mostly remains limited to books. Authorities in Nepal did little to promote science communication in the country to the next level. Many scientific organizations, government, and internationally funded projects established to promote science did little to expand the science communication network. Apart from organizing and hosting seminars, their engagement with the public at large remained limited. There are no dedicated shows on science or related material to trigger excitement in young kids and adults. Schools are running quiz shows and science exhibitions but are mostly urban-centric. The media still find space for sensationalizing any hysteria, hoaxes, or superstitions without thorough reporting which is counterproductive. The media support of efforts on the conservation of biodiversity in Nepal as grassroot campaigns was however hailed as a success to promote science communication and gained some traction. (https://blogs.scientificamerican.com/scicurious-brain/scicurious-guest-writer-societal-challenges-to-science-communication-in-nepal/

Lately, community radios in Nepal have used digital online portals and started podcasting science communication to reach out to marginalized listeners and extend their reach to those that prefer to receive their audio content from online platforms. Since April 2020, they started COVID-related information from verified sources and are now able to connect and share information with the world and unite the members living in different countries. The presence of community media on the internet will help minimize disinformation and fake news and build trust to take the information seriously.(Radio Madanpokhara podcast: http://bit.ly/3nLUcda, Radio Langtang podcast link http://bit.ly/3rm7tLx, Radio Kapilvaste podcast link http://bit.ly/3azlWy3, https://onlineradionepal.gov.np/en/2023/02/21/347304.html, https://www.unesco.org/en/articles/reaching-out-unreached-community-radios-nepal-take-podcasting

Nepal media initiative like Nepal Radio Education Teacher Training Project (RETT) for upgrading underqualified primary school teachers using radio techniques such as intensive broadcasting and, interactive learning with the help of USAID is helping teacher training and science communication, https://pubmed.ncbi.nlm.nih.gov/12315027/

Nepal celebrates National Science Day on Ashoj 1 (September 17) amidst various programs to highlight the progress the country made in science, technology, and innovation and help create a favorable atmosphere for the youths to study science and utilize their knowledge and skills. The government has initiated the science diplomacy dialogue and Science Journalism prize and expects that such an initiative will help raise public awareness of science and technology and science communication initiatives (Rastriya Samachar Samiti 2021 Kathmandu).

Both public and private media channels have grown exponentially in recent decades and is a growing industry. But in all of the media channels, science communication is a least priority especially in private media channels Radio, TV and internet and is dependent on sponsors.

Nepal Academy of Science and Technology (NAST) Science Popularisation Program

Following IDRC project, NAST has commenced following science communication and popularisation program (https://nast.gov.np)

1. Radio Program: First ever radio program on Science and Technology
2. Television Program: First ever television program dedicated to Science and Technology Publication
3. Vigyan Lekhmala: a popular science magazine in Nepali Language on contemporary issues in different fields of science and technology, distributed to school library media outlets to disseminate the information on science and technology to the general people.
4. NAST Communicator: A periodic bulletin in English language that covers the major NAST events
5. Popular Science Book Series: publishes queries raised by Science students and experts' response to the queries and aims to establish the culture of scientific curiosity in the society.
6. Booklets: On different scientific topics to make general people aware of the issues and very easy to understand for the common people.
7. Internal Circulation (NAST Sanchar): A weekly

bulletin of internal activities of NAST

8. Nepal Journal of Science and Technology: A peer review journal to provide a forum for Nepali Scientists and Technologists to publish their original research articles carried out in Nepal.
9. Proceedings of Conferences, Symposium, Seminars, and Workshops: A publication of proceedings papers presented in national and regional conferences, seminars and workshops organized by NAST.
10. Other Scientific Publications: Has published more than 150 major study reports, manuals, proceedings, research reports, occasional papers, popular science series, science feature for media, books etc.
11. Awards , Felicitations, and Fellowships:

Following are the list of annual awards given away by NAST.

a. The Chancellor Innovation Award: For making outstanding contribution in the field of Innovation in Nepal.
b. Science and Technology Academy Award: Conferred every two years for making remarkable contributions in the field of science and technology.
c. Nature Conservation Award: for outstanding contribution in nature conservation under any genre of science and technology.
d. Science Award: For extensive contribution in the field of science. It is conferred annually to scientists who have crossed 40 years of age.
e. Technology Award: For extensive contribution in the field of technology. It is conferred annually to the technologists who have crossed 40 years of age.
f. Youth Science and Technology Award: awarded to five young scientists below forty years of age, for endeavour and dedication in their field of expertise.
g. S&T Promotion Award: Awarded to two persons for promotion of S&T, and for creating public awareness on the importance of science and technology.
h. The World Academy of Sciences (TWAS) Award: for making remarkable contributions in Biology, Chemistry, Mathematics, and Physics.
i. NAST-Nabil Award: For making tangible contribution in the selected disciplines of Science and Technology
j. MD Basnyat Technology Academy Award: Awarded to an individual or institution for paramount contribution in research, especially in the field of energy.
k. JB Nakarmi Metalwork Award: for contribution on the metal related technology.
l. Bhubaneswor Low-Cost Technology Award: awarded to an individual or institution for the contribution on the low-cost technology.
m. Dayananda Bajracharaya Research Award: Awarded to best M.Sc. in Botany, Biodiversity, Biotechnology, Environment, Microbiology and Zoology.
n. Fanindra Prasad Neupane Research Award: for M.Sc. Thesis related to Plant Sciences.
o. Science Journalism Award: This Award is given annually to a journalist who makes outstanding contribution in propagating scientific news through different media.
p. Woman Scientist Awards: awarded to individual female scientists for her extensive contribution in the field of science.
q. Science Teacher Award: Awarded to science teacher for science teaching in schools.
r. Science Students Award: Awarded to best two students for securing the best result in science stream in the grades 11 and 12.

Apart from the above awards, NAST also established awards for outstanding personalities who contributed in laying the foundation of Science and Technology in Nepal. NAST also supports Nepali Scientists, Technologists or Innovators providing following different Grants and Fellowship schemes.

Research and Travel Grants: Awarded to researchers and innovators for research activities or innovations and to attend the scientific events. NAST also provides M.Sc. Thesis grant for promising proposals in different disciplines of Science and Technology.

Fellowship: One-year fellowship such as Brain-pooling Nepal fellowship, Research Fellowship, Assistant Research Fellowship and Research Assistantship

outstanding Ph.D. candidate fellowship.

NAST honorary fellowship: NAST Eminent Fellow, NAST Associate Fellow, and National Researcher Identity is awarded to the prominent Nepali Scientists or Technologists working in Nepal or abroad for their outstanding achievements.

Science Communication Campaign: Since its inception the academy has been actively involved in promoting public understanding in science and technology through various science popularization programs. The NAST is so far the only institution in Nepal that has been conducting regular programs to popularize science and technology among the people. In 1985, the academy launched a Science Popularization Project with grant support from the International Development Research Centre (IDRC), Canada. The objective of the project was to enhance science literacy among general people by utilizing mass media channels. This was the first systematic attempt to make the attributes of science and technology accessible to the general public in Nepal. Science popularization programs are now fully operated by the academy with its own resources. These programs include organization of science exhibitions, interaction and lecture programs, publication of science magazines and journals.

Science Fair: Science fairs are organised in different parts of the country, mostly in collaboration of local schools. The objectives of these programs are the increase public awareness on the importance and usefulness of science and technology in people's daily life and cultivate a scientific temperament among young people. The programs include a wide range of participatory activities for the high school students such as Competitive Science and Technology Exhibitions (High Schools Level), Science and Technology Quiz, Oratorical Contest, Essay Competitions, Science Teachers' Workshops/Training, Lecture Programs, Public interactions, Environment Camp (Occasionally), Science and Technology Film show. For greater participation of school children and the general public, these activities are tied up with local cultural and sports events.

Scientific Events: Periodic scientific events such as Science Fair, Trainings, Workshops, Seminars, Talk programs, Science Dialogues, national and regional science conferences, international events in Kathmandu as well as in different parts of the country are organised regularly.

Scientists' Forum: NAST has formed Women Scientists' Forum and Young Scientists' Forum to emphasize the roles of women and youth in Science and Technology for future.

Nomination to participate in international flora: To potential candidates of NAST or other institutions of the country to participate in different activities conducted by the institutions, societies, or academies related to Science and Technology in different parts of the world.

When the government of Nepal designated a separate ministry to take care of Science and Technology of the country in 1996, the Ministry also conducted similar activities. The B.P. Koirala Planetarium, Observatory, and Science Museum is an institution under MoEST that conducts science popularization activities such as producing television programs, publication of journal, organize training and other events related to science popularization, show scientific movies in it 3-D theatre, open science museum and observatories to all interested ones, etc. Different institutions under the universities of Nepal and other non-governmental institutions are also aggressively involved in science popularization missions in Nepal. The list of successful institutions involved in formulating the foundation of science and its popularization through different types of services in Nepal are summarized in Table 1.

Table 1: Following is the list of institutions involved in formulating foundation of science and its popularization through different types of services in Nepal.

SN	Estd. Year (AD)	Name of the Institution
1	1890	Prithvi Bir Hospital
2	1894	Bell Tower
3	1901	Gorkhapatra Press
4	1911	Pharping Hydroelectric Power Plant (Chandrajyoti Hydro-electric power station)
5	1911	Bijuli Adda/Dept. of Electricity (1962)

6	1913	Telephone Line Distribution, Trunk Call (Ktm to Raxaul)in 1914,
7	1916	Automobile
8	1918	Trichandra College
9	1922	Ropeway (22 km), Extended to 42 km (1964)
10	1924	Agriculture Office
11	1927	Railway Service
12	1932	Technical School
13	1934	Civil Medical School (Paramedics)
14	1936	Biratnagar Jute Mills
15	1937	The Juddha Fire Brigade
16	1939	Department of Livestock Services
17	1942	Training School for Sub-Overseers
18	1942	Forest Training Center for Rangers
19	1947	Nepal Forestry Institute
20	1948	Department of Publicity, Department of Information and Broadcasting
21	1950	First Aeroplane Landing
22	1951	Radio Nepal
23	1954	Engineering School
24	1955	Department of Forest
25	1956	Department of Soil Sciences
26	1957	School of Agriculture, College of Agriculture (1968)
27	1957	Survey Department
28	1958	Department of Geology and Mines (DHM)
29	1958	Department of Survey
30	1958	Central Bureau of Statistics (CBS)
31	1959	Tribhuvan University (TU)
32	1960	Department of Medicinal Plants
33	1960	Botanical Survey & National Herbarium
34	1960	Balaju Yantra Shala
35	1961	Department of Food (1961), Food Research Lab (1966)
36	1962	Department of Hydrology and Meteorology (DHM)
37	1963	HMG's Press, Dept. of Printing and Publication (1988), Dept. of Printing (1992)
38	1963	Butwal Technical Institute
39	1963	Forest Survey Research Office
40	1964	Royal Drug Research Laboratory (1964)
41	1965	Janakpur Cigarette Factory (Estblished with Russian Support) Closed in 2011
42	1965	Postgraduate Departments of Natural Sciences, TU
43	1968	Lumle Agriculture Research Centre
44	1968	College of Agriculture
45	1970	Department of Roads
46	1970	Department of Buildings
47	1971	Ministry of Communication

48	1971	First Computer in Nepal (2nd Generation IBM-1401) for National Census
49	1972	Royal Drugs Limited
50	1972	Institute of Medicine, TU
51	1972	Institute of Agriculture and Animal Sciences, TU
52	1972	Institute of Forestry, TU
53	1972	Institute of Engineering, TU
54	1973	Department of National Parks and Wildlife
55	1974	Himal Cement Factory (First Cement Factory in Nepal), Closed in 2002
56	1974	Department of Soil Conservation & Watershed Management
57	1974	Electronic Data Processing Centre (EDPC), National Computer Center (1980)
58	1975	Trolley Bus (Tripureshowor to Suryabinayak, 13 km), Ended in Nov 2008
59	1975	Nepal Telecommunication Corporation, Nepal Telecom (2004)
60	1975	Agricultural Project Research Center (APROSC)
61	1975	Natural History Museum
62	1976	National Council of Science and Technology (NCST)
63	1976	Nepal Institute of Standards (1976), Nepal Bureau of Standards (1981)
64	1977	Research Centre for Applied Sciences and Technology (RECAST) TU
65	1980	Department of Drug Administration (DDA)
66	1980	Department of Agriculture Development
67	1981	Water and Energy Commission
68	1981	Herbs Products and Processing Company Limited
69	1982	Nepal Academy of Science and Technology (NAST, 2007)
70	1982	National Trust for Nature Conservation (NTNC)
71	1982	Department of Ayurveda
72	1983	International Centre for Integrated Mountain Development (ICIMOD)
73	1983	Nepal Forum of Environment Journalists (NEFEJ)
74	1985	Nepal Television
75	1985	National Forensic Laboratory
76	1985	Bhrikuti Paper Mills, Privatized with Golchha Org. (1992), Closed in 2011
77	1986	Research Laboratory for Agriculture Biotechnology and Biochemistry (RLABB)
78	1986	Resources Himalaya Foundation (RHF)
79	1986	Mahendra Sanskrit University
80	1988	Department of Irrigation - Department of Water resources and Irrigation
81	1989	Centre for Rural Technology (CRT)
82	1989	Centre for Technical Education and Vocational Training (CTEVT)
83	1990	Centre for Environmental and Agricultural Policy Research
84	1991	The Pyramid Laboratory
85	1991	Kathmandu University (KU)
86	1991	Nepal Agricultural Research Council (NARC)
87	1991	Nepal Health Research Council (NHRC)
88	1991	Centre for Renewable Energy
89	1991	Department of Water Induced Disaster and Prevention

90	1992	B.P. Koirala Memorial Planetarium, Observatory and Science Museum
91	1992	Gorakhkali Rubber Industry (GRUL), Halted Production in 2014
92	1992	Environment Protection Council
93	1992	Nepal Environmental and Scientific Services (NESS)
94	1993	B.P. Koirala Institute of Health Sciences, upgraded to deemed university (1998)
95	1993	World Wildlife Fund (WWF), Nepal
96	1993	Department of Electricity Development
97	1994	Purbanchal University (PU)
98	1994	Nepal Engineering College (NEC)
99	1994	Manipal College of Medical Sciences
100	1995	Safa Tempo (Electric three-wheeler public vehicle)
101	1995	Ministry of Population and Environment
102	1996	The World Conservation Union, IUCN
103	1996	Ministry of Science and Technology (MoST)- Ministry of Env,S&T -MoST(2009)
104	1996	College of Medical Sciences, Bharatpur
105	1996	High Level Information Technology Commission
106	1996	Alternative Energy Promotion Center (AEPC)
107	1997	Pokhara University (PoU)
108	1997	Kathmandu Medical College
109	1997	Nepal Medical College
110	1997	Nepalgunj Medical College
111	1997	Nepal Telecommunication Authority
112	1998	Kathmandu Engineering College
113	1998	Kantipur Engineering College
114	1999	Centre for Energy Studies (CES), TU
115	2000	Dept. of Food Technology and Quality Control (DFTQC)
116	2000	Shikhar Biotech Co. Ltd.
117	2001	National Information Technology Center/ Government Integrated Data Center
118	2001	National Agriculture Research and Development Fund (NARDF)
119	2002	National Academy of Medical Sciences, Bir Hospital
120	2003	Information Technology Park, Banepa
121	2003	Real Time Solutions Pvt. Ltd.
122	2004	Nobel Medical College
123	2004	Lumbini Buddhist University
124	2004	Nepal Development Research Institute (NDRI)
125	2006	Technology Sales Pvt. Ltd., RamLaxman Innovations (Currently)
126	2007	Centre for Molecular Dynamics, Nepal (CMDN)
127	2007	National Information Commission
128	2008	Patan Academy of Health Sciences
129	2009	National Ayurveda Research and Training center (NARTC)
130	2010	Mid-Western Univeristy
131	2010	Far-Western University

132	2010	Agriculture and Forestry University
133	2011	Karnali Academy of Health Sciences
134	2011	Research Institute for Bioscience and Biotechnology (RIBB)
135	2012	National Innovation Center
136	2014	Kathmandu Institute of Applied Sciences (KIAS)
137	2015	Pokhara Academy of Health Science
138	2015	Center for Health and Disease Studies-Nepal (CHDS-Nepal)
139	2016	Nepal Open University
140	2017	Rajarshi Janak University
141	2017	Rapti Academy of Health Science
142	2018	Global Institute for Interdisciplinary Studies (GIIS)
143	2018	Nepal Applied Mathematics and Informatics Institute (NAAMI)
144	2019	Gandaki University
145	2019	Madan Bhandari University Science and Technology

Table 2: List of Overseas Funding Agencies

- Science Popularization Project ((IDRC, Canada)
- Teachers' Training (USAID)
- Radiation Laboratory establishment at NAST (USAID)
- Energy Efficiency Program (GIZ)
- Promotion of Renewable Energy Technology (GIZ)
- Disaster Management Practices (UNDP)
- Modernization of Traditional Technologies (UNDP)
- Achieving Sustainable Development Goals (UNDP)
- Nepal Climate Change Support Programme (UNDP)
- Seismic Vulnerability Mapping (UNDP)
- Post- 2015-Earthquake Reconstruction (UNDP)
- Technical and Vocational Education and Training (UNDP)
- Enhancing policy, planning and monitoring to achieve the targets of Education (UNESCO)
- Science and Technology National Database Updating (UNESCO)
- Prioritization in Science and Technology (UNESCO)
- Training on Traditional Building Architecture and Bylaws (UNESCO)
- Support for STI policy formulation (UNESCO)
- Protecting Nepal's natural heritage (UNESCO)
- Communication and Information, Safety of Journalists (UNESCO)
- Education for All (UNESCO)

- Awards, Fellowships, Trainings (TWAS)
- Region office for Tuberculosis in South East Asia (WHO)
- National Immunization Program (WHO)
- Science Awareness, Training and Workshop (IAP)
- Participation in Trainings, Seminars, Workshops (NAM S&T Center)
- Nature Conservation (WWF)
- Rainwater Harvesting (UN-Habitat)
- Biogas Support Program (FAO)
- Water, Sanitation and Hygiene, WASH (UNICEF)
- The Economic Viability of Jatropha Biodiesel in Nepal (World Bank Group)

5. Science Communication Approach and Role of Media in South Asia and Global Trend

Science communication priorities and needs are different for developed and developing countries. While developed countries are debating environmental effects, ozone layer depletion, genetically modified food as a priority issue, developing countries' priorities remain providing potable clean water, health and hygiene, conservation of energy etc. In developing countries, even non-illiterate people are inquisitive for evidenced based information on issues affecting their livelihood, but there is a lack of an attractive, catchy and effective communication to the level of their understanding. In developing countries, science communication has to compete to get the media coverage against political, crime, sports or business and films / entertainment, religious and superstitious programmes but experts believe this can be addressed by making science communication saleable by pitching on value-based information and subject matter of public interest.

Developed countries have institutionalised science communication initiatives with innovative approaches by creating new science communication avenues and online platforms which includes a range of public speaking, writing, journalism and social media initiatives and offer various fellowship and support to science writers. Science communication degree courses in the Universities are not limited to formal lectures anymore and use innovative formats, such as comedy, using props, powerpoint Karaoke or the form of a story and focuses on addressing gender and racial inequity. One of the hurdles is still lack of funding for science communication training. It is recommended that all science projects should set aside some funding for communicating science to the general public of their findings. In Canada, freelance science writing launched a podcast (e.g. Broad Science) and tried innovative formats (such as Wikipedia Edit-A-Thons) or utilizing your social media platforms (like @science.sam) to share exciting science news and related stories (Farah 2009).

An excellent review paper is published on science communication strategy and authors are referred to the article for details (Bubela 2009). Public communication for Science and Technology (PCST), an international network devoted to science communication has announced it will set up an academy to promote science communication, says Vladimir de Semir, chair of PCST (the International Network on Public Communication of Science and Technology). The academy plans to raise funds to support the network's activities in the developing world. It will also operate a regularly updated website that will gather information on science communication (Yang 2007).

In Asia, SAARC countries (Patairiya 2007; Goh 2008) are still behind from awareness of basic issues like clean drinking water, health and hygiene, conservation of energy and there exist a wide gap between scientific knowledge and community social needs. India is now considered one of the powerhouses of innovation and had set science communication strategy as early as 1987 with Bharat Jan Vigyan Jatha program BVJP. India has developed a number of outreach programmes through folk forms, digital media and hands-on activities like popularisation of HAM, Radio, origami and astronomy for different target groups teachers, students and

marginalised people with low level of literacy in science communication. The Bangladesh Council of Scientific & Industrial Research (BCSIR) has also introduced a program to develop social relations of scientists with the public, government, other organisations, foreign countries, and UN bodies, and publication of booklets, leaflets on various scientific discoveries and innovations.

The National Science Foundation (NSF) of Sri Lanka has a slightly different approach and its programmes are aimed at developing effective utilisation of science in daily activities, and developing understanding of science and technology to renew traditional beliefs, customs and practices in contrast to India's approach against superstitions and misbeliefs. Science programme for public understanding has been initiated under the theme 'Science for All' to raise science literacy at community level to benefit and appreciate the value of science communication. The NSF organises science magazines on TV, publication of Vidurava magazine, but in contrast science still remains an alien subject for common man in the developing world . Pakistan has adopted 'deficit model' of public communication of science and technology, which focuses to help people acquire knowledge of modern science and most of the science popularisation programmes science museums, planetariums, and mobile exhibitions, etc., but targeting mainly the literate audience.

Based upon individual countries experiences, the observations and analysis of the data available through various sources, following recommendations are being considered for an effective science communication in the region which broadly includes: Developing Regional network, South Asia Science Communication Forum, use of regional languages in science communication, identify strengths and best practices of every state, Govt and media commitment to support science institutions to train science communicators and science writers/ journalists/scientists, commitment to the goal of science communication as a unifying source for all member states, formation of Science communication wing as part of SAARC S&T Committee, devise exchange programme for students, science communicators, scientists and journalists in member states, regional science communication awards to encourage talented science communicators in the region, annual event, like regional science festival or congress, Joint regional training workshops on various aspects of science communication/science writing/science journalism, harnessing support from governments of member states and international organisations.

There were some notable achievements such as the recent agreement for S&T cooperation between some of the countries in the region, an increased participation of cross-national scientists, journalists and science communicators in various PCST activities and programs and are hailed as milestones achievements. Efforts to run India's Vigyan Rail / Vigyan Mail (Science Train: An S&T Exhibition on Wheels) also in Pakistan and Bangladesh to various destinations during 2004-2005 has attracted millions of people. SciDevNet, a UK based science and development web network, has started its South Asia Regional Gateway [www.scidev.net] incorporating information on science and development subjects of the region, thereby offering stronger science communication. India's Annual National Science Communication Congress and National Children's Science Congress offer forums for participation and exchanging views and experiences of scholars and students interested in PCST. However, it may be the rosy side of the picture and still there are many more miles to go together to achieve the desired level of public understanding of science; the countries in the region have to come closer for the purpose. This is only the beginning! We may look forward to better cooperation in PCST activities in the SAARC region in the years to come.

6. Non-Resident Nepali Media and Science Communication

Non-resident Nepalese Association (NRNA, www.nrna.org.np) which represents the largest Nepalese community representing 4.5 million Nepalese living overseas in 86 different countries has their own media channels, online and social media presence and impart influence on Nepali media. While the organisation is developing a long-term strategy for them in the S & T investment and Science Education campaign, science communication is not a top priority. One of the flagship projects of NRNA SKI Open University initiative [https://en.wikipedia.org/wiki/Nepal_Open_University] was to provide skill and science-based education outreach and effective science communication to the marginalised population of Nepal and was a welcome move. Nepal Science Foundation Trust (NSFT) also aims to promote science awareness campaigns through the "Science for the People" programs and assist in science capacity building, and lobbying at the policy level. Its science communication strategy

included developing interaction programs with science teachers in schools, Science fellowships for media, and science journalism award supporting science education campaigns. The initiatives also included environmental awareness study visit programs and promotion of the World science day activities and Science Olympiad (Fig. 1). Brain Drain seminar, a joint initiative with BSN and NAST also aimed to create an interactive forum between Nepalese and Diaspora's professionals to share R&D experiences and networking and support

Fig. 2 : NSFT core activities and Jurie Landslide and environment impact study visit and World Science Day (Source: NSTF)

science communication.

NRNA biannual Global Knowledge Convention main focus is also on application of natural sciences, advocating science policy , science education and communication. NRNA global conference [https://nrna.org] has provided key recommendations to science education programs and includes establishing an innovation endowment fund in partnership with the Ministry of Education, Science and Technology and NAST, and increased budget for science education and science communication. NRNA is also supporting the establishment of a science learning centre at NAST premises which aims to create a national forum for a science awareness campaign and communication.

7. Barrier in Effective Communication

There are still many barriers to effective communication to the general public. It requires many years of training and practice to make specialized knowledge and technical terminology understandable by the public. For example, climate change and pandemic issues have been communicated widely with a list of policies and activities for mitigating climate change and spreading the message of zero emission by 2050. However, it has created confusions while raising awareness about the issue due to conflicting news from both supporters and denial groups. Communication materials should be based on evidence as people interpret new information in light of their perceived beliefs, referred to as their

mental models. In science communication one of the approaches has also been to conduct public surveys but such feedback needs to be statistically significant with larger sample sizes. The feedback questions need to be designed to measure how well people know and understand the facts for making informed decisions.

The mental model's approach in communications recognizes that people need information that not only addresses their knowledge gaps and misconceptions but also builds on their existing beliefs. There is a need for interdisciplinary research involving scientific experts, social scientists and media experts, to promote development of more effective communication materials about scientific topics relevant to the general public. The resulting evidence-based communications are more likely to address what people need to know to make more informed decisions, allowing them to obtain better outcomes for themselves and the society in which they live.

8. Challenges and Opportunities

The history of the popularisation of science is driven by the philosophy that science is for the benefit of the people and to learn, safeguard, and make our universe most liveable. The world's diversity of cultures and level of education access and its assimilation is different . In developing countries like Nepal, modern science and technology and media both have failed to play a meaningful role in addressing the pressing needs of improved nutrition, drinking water, public health, safety, and shelter especially in regional and rural areas and in marginalized populations. The investment in S&T and science education had remained a low priority until today. Despite this, in the last two-decade, Nepal's connectivity to the world has increased exponentially even in remote parts of Nepal through access to smartphones and wireless internet. The people at all levels hold a mobile phone as a necessity and are now well informed and connected in all parts of the country. They are also connected to social media FB, WhatApps, Viber, TikTok, Instagram and have learned to realize how science can be helpful to their needs and other countries have benefited from the advancement and application of science. However, they are not aware of the investment and commitments countries have made to reach this stage and why Nepal failed to commit to science education. The perception of science in Nepal is still a complex subject and experimental driven which needs to be removed and awareness programs about the importance of science in daily life and science as fun and popular subjects need to be launched.

It would require much more concerted efforts to feed popular science communication to the public and especially children. The major competitors of science coverage in public are news and news-based programs which focus on political, crime, sports or business, drugs, violence, sex, films/entertainment, and now religious and superstitious programs dominate and science always remains in backseat getting almost insignificant attention. This situation can be changed by making science a saleable product through fun experiments, Quiz competitions, science exhibitions, talents programs, online teaching of popular science topics, interaction with scientists, sharing discoveries in print features, radio/TV programs, and the Govt should allocate a budget for all science institutions and schools. The private industry should also be encouraged to share their product's invention story alongside the advertisement. If a science story is told with a story and journalistic flavour, based on evidence, and flavoured with spicy examples and presented with vibrant dynamism by school children, celebrities from different fields such as cinema, sports, politicians, CEOs of the company, people will be attracted to science. There is a strong need to make science communication simpler and more attractive; and the real challenge is to stay focussed on literacy, practical, civic, and cultural aspects of science. The debates about important topics such as nuclear power or genetically modified foods, greenhouse, pandemic crises should also happen at the community level. Developing countries need to develop the mechanism for creating public debate that invites and involves people's participation and make them understand the impact of this confronting and emerging issues and the impact of science and benefit in day-to-day life. Otherwise, science still remains an alien subject for the common man in the developing world and is confined to the lab and elite scientific community. The awareness of basic issues like providing potable water, health, and hygiene, conservation of energy should be the priority areas for science communicators in the developing world. Also, what is important is an emphasis to produce a science program which is interwoven with elements of entertainment and public interest.

World science day should be declared a holiday. Scientists should be the real idols in real life for their real contribution to improving people's lives and making this world a better place. The life story

of Nobel laureates should be shared to inspire young generations and how their discoveries have benefitted human mankind, Scientific research, and discovery is described in the literature and is the foundation of future inventions and discoveries and should be explained in science teachings in secondary schools. Highlighting scientists and their work is one way, to re-foster trust in science and communicate to young people the importance of their contributions and set role models for doing public good (Fouad Laroui 2020).

UNESCO's initiatives on inclusive policy lab, open science, science education, and advocacy for science, are great and are aimed to take science to the grassroots to raise awareness about the pressing challenges like climate change, poverty and pandemic crises. Environmental conservation, biodiversity, and global warming narratives, and natural ecosystems and Nepal's rich geo-climatic and importance of mountains should be taught online using simple models to connect the science to students and community. Science popularisation can only be achieved by communicating success stories. Modern digital technology and social media connectivity is a great tool and platform to advocate and popularise the benefit of science. UNESCO should also translate their program into different languages in different countries and use visual media. Science popularisation can only be achieved by communicating success stories. Science and belief can co-exist. *Science is not the enemy of religion (UNESCO 2021).*

9. Conclusion

Despite increased initiatives, in the past two decades, Nepal's priorities for science campaigns are still very low and have failed to bring any visible impact or change the public perception of the importance of science education and S&T. Science still remains an alien subject for common man in the developing world. Government S&T funding has remained poor, barely 0.45% of GDP during the last six decades. By 2025, 40% of global employment in any industry would need at least an undergraduate level manpower and with the current priorities, Nepal is likely to fare poorly in this arena.

Twenty percent of the marginalized population still do not have access to basic education and are not able to understand science-based information. The World Trade Organization (WTO) has predicted that by 2020. Nepal needs to drastically increase the budget for science education and research proportionately and fast track S&T infrastructure development to benefit from science and join the global S&T race. Nepal S&T institutions and universities urgently need to commit to long-term strategies on science education campaigns, develop policies, priorities, and financial commitments, and make science education mandatory in school education to meet its millennium goals. Media has a greater role to play in championing the science education cause. Journalism diploma and degree should also include science journalism courses and will play a key role in advancing the cause of the science education campaign. Professional Societies also need to play a proactive role and join in this campaign. The NAST initiative to set up a National Science Park is a welcome step and such a park should be established in every state.

The NRNA and diaspora community can play a meaningful role to advance the cause of science education. There is a strong need to set up a joint innovation science education endowment fund between NRNA and the Ministry of Education, Science and Technology, GoN, Nepal Academy of Science and Technology (NAST) and develop a white policy paper on science education and communication in the next 5 years.

Recently PM Dahal reiterated his government's commitment to supporting the development of science and technology and it is hoped that science communication is included in this policy framework. The government needs to develop a national science communication policy and provide funding to bring and unite the efforts of Media, Industry, Scientific, and educational institutions both on and offline science communication.

Science education is inevitable in the 21st century. It is the root of knowledge which drives innovation and empowers people and countries with creativity to create new opportunities, address any pandemic and natural disaster crises to a path of prosperity. Science knowledge must be disseminated effectively to make people understand the value of science knowledge, increase interest, wider participation and society overall to benefit. Nepal must realize that time is running out for them to join the global race of knowledge power and science communication is one of the fundamentals to be part of this knowledge ecosystem. The country should make science education mandatory in schools and provide necessary infrastructure and connectivity to the

global knowledge pool to skill and reskill our human resources.

References

Bajracharya, A., W. Brouwer, 2007. A narrative approach to science and teaching in Nepal, *International Journal of Science end Education*, 429-446.
DOI: 10.1080/0950069970190406

Bubela, T., M. C. Nisbet, R. Borchelt, F. Brunger, C. Critchley, E. Einsiedel, G. Geller, A. Gupta, J. Hampel, R. Hyde-Lay, E. W. Jandciu, S. A. Jones, P. Kolopack, S. Lane, T. Lougheed, B. Nerlich, U. Ogbogu, K. O'Riordan, C. Ouellette, M. Spear, S. Strauss, T. Thavaratnam, L. Willemse, and T. Caulfield, 2009. Science communication reconsidered, *Nature Biotechnology* volume 27, 514–518.
DOI:10.1038/nbt0609-514

Farah, 2009. A Beginner's Guide to Science Communication Opportunities in Canada https://farahqaiser.medium.com/scicomm101ca-99395f2cee63

Goh, B., A. Pomsagun, M. Le. Tissier, W. C. Dennison, H. H. Kremer, and J. Weichselgartner, 2008. Training Workshop on Science Communications for Southeast Asia / South Asia, LOICZ Reports and Studies No. 31, LOICZ International Project Office GKSS-Research Center.

Gimeno, C., 2019. What makes a good communication, dissemination and exploitation plan of a research project? Part 1 – Communication". Leitat Projects' Blog. Laroui, F., 2020. https://indiaeducationdiary.in/building- trust-in-science-essential-to-the-...

Nepal Education Since 1951, The library of Congress Country Studies, CIA World facebook.

https://photius..com/countries/nepal/society/nepal_society-education_since1951.html

https://onlineradionepal.gov.np/en/2023/02/21/347304.html

https://www.unesco.org/en/articles/reaching-out-unreached-community-radios-nepal-take-podcasting

https://pubmed.ncbi.nlm.nih.gov/12315027/

https://blogs.scientificamerican.com/scicurious-brain/scicurious-guest-writer-societal-challenges-to-science-communication-in-nepal/

IDRC Report 1992.

Nepal Academy of Science and Technology, https://nast.gov.np

Patairiya, M., 2007. Challenges and Prospects of Science Communication in South Asia, *Indian Journal of Science Communication*, Vol. 6 (1), 9-16.

Roberts-Artal, L., 2015. A brief history of Science Communication, https://blogs.egu.eu/geolog/2015/02/06/a-brief-history-of-science-communication

Radio Madanpokhara podcast: http://bit.ly/3nLUcda

Radio Langtang podcast link http://bit.ly/3rm7tLx

Radio Kapilvaste podcast link http://bit.ly/3azlWy3

Rastriya Samachar Samiti 2021 sep 14, 13:01, Kathmandu.

Roberts-Artal, L., (2014). GeoEd: Citizen Geoscience,

https://blogs.egu.eu/geolog/2014/12/05/citizen-geoscience

https://en.wikipedia.org/wiki/Nepal_Open_University

UNESCO recommendation on open science., (2021) SC-PCB-SPP/2021/OS/UROS, https://unesdoc.unesco.org/ark:/48223/pf0000379949.locale=en

Yang, J. Ou., 2007. *Technical Task Force: A Pilot Project of Agricultural Science Communication in China*, Indian Journal of Science Communication, Vol. 6 (1), 3-8.

Electronic Properties of Semiconducting Nanowires: a Comparative Study

Sanjay Prakash Kaushik[1*], Manoj Kaushik[2], Satyendra Singh[3], Ram-Krishna Thakur[4] Vivek Kumar Shukla[5] and Vijay Kaushik[6]

[1&2]State Council of Educational Research & Training, Gurugram, Haryana-122001, India
[3]Department of Physics, Seth G. B. Podar College, Nawalgarh, Rajasthan -333042, India
[4]Research and Academic Affairs, JNU Jaipur, Rajasthan-302017, India
[5]Department of Physics, Gautam Budha University, Greater Noida, U.P.-201312, India
[6]Department of Science, Scottish High International, Gurugram, Haryana – 12200, India

***CORRESPONDING AUTHOR:**

Sanjay Prakash Kaushik
Email:physicistkaushik@gmail.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI :
https://doi.org/10.3126/njst.v21i2.67168

Date of Submission: Mar 10, 2023

Date of Acceptance: Mar 19, 2024

ABSTRACT

The presented work has explored the comparative study of electronic properties of semi conducting nanowires of different materials. These nanowires have critical role in photovoltaic and it shapes the future of new and renewable energy. The study has been performed for different shapes of nanowires. The various shapes under consideration are 2-atom Linear Nanowire, 2-atom Zigzag Nanowire, 4-atom Square Nanowire and 6-atom Hexagonal Nanowire. The findings for electronic properties reveal that 2-atom linear wire can be conducting as well semi conducting, 2-atom zigzag wire is conducting for almost all materials, 4-atom square wire is insulating for most of the materials while 6-atom hexagonal wire has come out to be insulating for all materials. Hence a semiconducting material shows conducting, semiconducting and insulating behavior depending on the proposed shape for various materials.

Keywords: Nanowire, Electronic properties, Density functional Theory, Pseudopotential, Band structure.

1. Introduction

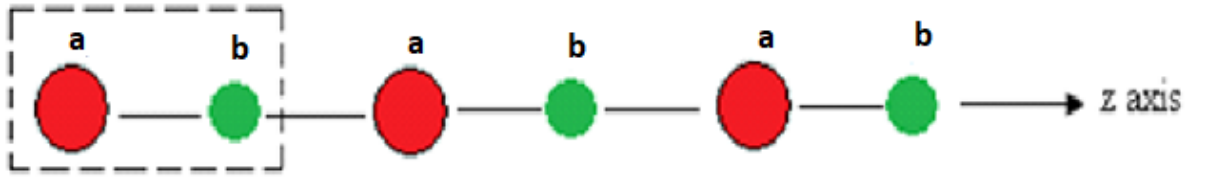
The present era is era of Science and Technology. In this technological era the behavior of electronic devices matters a lot. The photovoltaic has played its critical role in the present industrialization by bridging the gap between the demand and supply of ever-increasing demand of electricity. The efficiency of the photovoltaic cell is dependent on the semiconducting wire used for its manufacturing. The presented work revolves around this central idea of efficacy of photovoltaic cell and has explored the electrical behavior of semiconducting nanowire which are used in solar cells and has also explored other potential material for the same purpose. The electrical behavior of semiconducting nanowires plays a very critical role for the desired output efficiency of such electronic devices. The material like cadmium sulphide, zinc selenide and cadmium telluride in particular play a critical role in efficiency of solar cell.

Many researchers have shown their keen interest in the electrical properties of the nanowires since around 1980 and have come out with significant conclusions. If we compare the bulk form with the

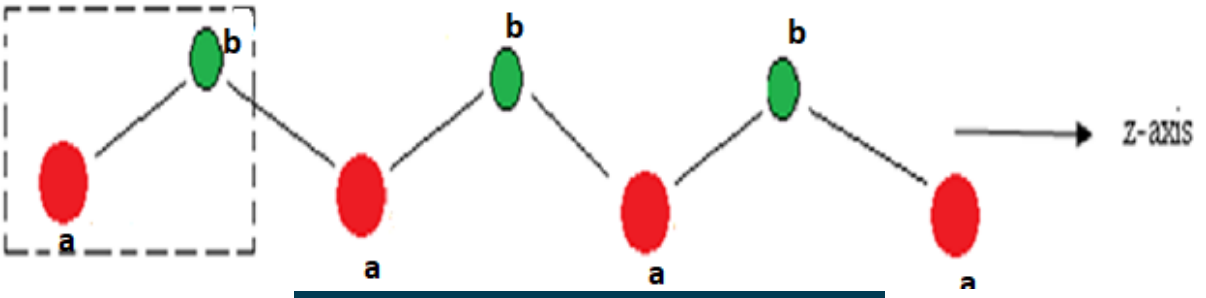
nano dimension of these materials then a variation in electrical as well as other properties is noticed. Exotic properties were reflected by these miniature structures at nano-scale and because of this there has been an urgent need for exploration and investigation of such structures. The devices made on this technique are seen to have technical advantages over other devices based on photolithography. The Review on structural dependence of electronic behavior of various nanowires as reported by the researchers in past around thirty years is the outcome of this proposed work.

From the early eighties of 20th Century, the germanium and silicon nanowires have been the central spot for the researchers and thus sufficient experimental work on these nanowires have already been performed by various researchers (Li *et al.* 2003; Tian 2007; Greytak 2004). The work has explored review of electronic properties of various semiconducting materials as proposed by many researchers and it also includes our work in which we have performed the study for cadmium sulphide, zinc selenide and cadmium telluride. Cadmium Sulphide is used as buffer layer in the cell (Kapadnis *et al.* 2020) and is also used in designing the photovoltaic cell, Zinc Selenide, which may be used to improve the absorption coefficient which affects solar cell (Nisreen *et al.* 2020) and Cadmium Telluride which can be used for increasing the efficiency of Solar Cell (Fardi *et al*. 2013). The semiconducting nanowires play a significant role in efficiency of present-day devices and hence are the material under much explored study. This has attracted us to explore these materials in terms of electronic properties and hence we have performed the review which shall be very critical for future researchers.

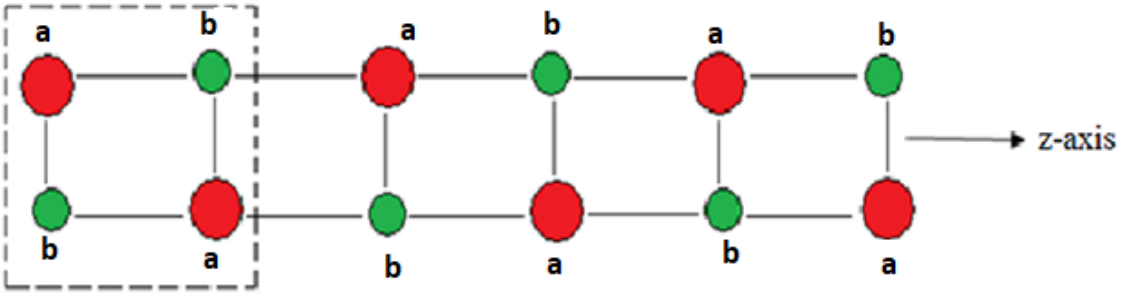
The different atomic arrangements are discussed in the performed study. The various shapes explored by the different researchers in this review are: 2-atom linear, 2-atom zigzag, 4-atom square and 6-atom hexagonal shapes as shown below in Fig. 1.



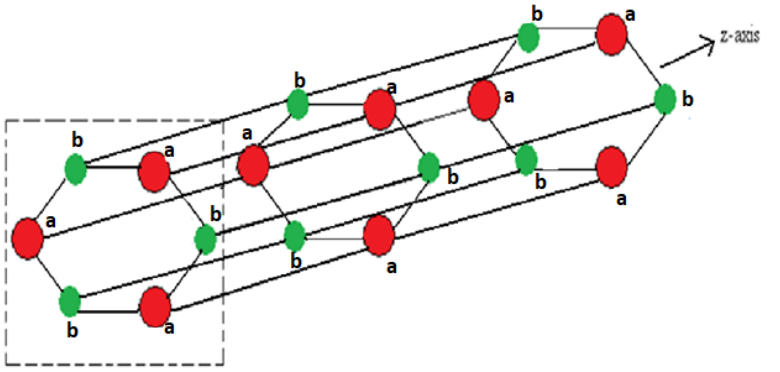
(a) 2-atom Linear Nanowire



(b) 2-atom Zigzag Nanowire



(c) 4-atom Square Nanowire



(d) 6-atom Hexagonal Nanowire

Fig.1. Structures of Nanowires (a) 2-atom Linear Nanowire (b) 2-atom Zigzag Nanowire (c) 4-atom Square Nanowire (d) 6-atom Hexagonal Nanowire.

Srivastava (Srivastava *et al.* 2008) studied Gallium Nitride by pseudopotential density functional by using generalized gradient approximation (Perdew *et al.* 1996) and proposed that the 2-atom linear wire reflected semi conducting nature, 2-atom zigzag & 4-atom square wires reflected metallic nature and 6-atom hexagonal wire showed insulating nature. Materials at lower dimension show different behavior for Gallium Arsenide was established by a group of researchers and it was also proposed in their findings that for 2-atom linear and 2-atom zigzag shapes are conducting for GaAs whereas 4-atom square and 6-atom hexagonal shapes are insulating (Singh *et al.* 2009). The same group of researchers employed ab-initio DFT calculations (Hohonberg *et al.* 1964; Kohn *et al.* 1965) on Gallium Antimonide and proposed that the 2-atom linear and 2-atom zigzag shapes are conducting here also whereas 4-atom square and 6-atom hexagonal shapes are insulating in nature for GaSb. In 2011, Srivastva (Srivastva *et al.* 2011) used exchange correlation potential of Trouiller– Martin (Troullier et al. 1991) and performed the study on Gallium Phosphide and proposed that the 2-atom linear & 2-atom zigzag wires are reflecting metallic nature whereas 4-atom square and 6-atom hexagonal wires are insulating in nature. Srivastva (Srivastva *et al.* 2011) performed the structural dependence of aluminum nitride and predicted the electronic behaviour of the material by stating that 2-atom linear, 2-atom zigzag & 4-atom square shape is conducting in nature whereas 6-atom hexagonal wire have shown insulating character. Singh (Singh *et al.* 2015) by employing 15-k point sampling for integration of Brillouin Zone by Monkhorst-pack method (Monkhorst *et al.* 1976) on Zinc Oxide (ZnO) performed the ab-initio study for electronic behavior, his study for band structure indicated that 2-atom linear shape is semiconducting, 2-atom zigzag shape is semiconducting while 4-atom square and 6-atom hexagonal shapes are insulating in nature.

We have also performed the ab-initio DFT calculations and decided to extend the work for II-VI semiconducting nanowires and choose Cadmium Sulphide (CdS), Zinc Selenide (ZnSe) and Cadmium Telluride (CdTe). The selected wires have their unique applications like ZnSe semiconductor has its unique application as light emitting diodes (Chen *et al.* 2005), photo detector (Vigue *et al.* 200) & scintillator (Nasieka *et al.* 2014) while CdS semiconductor has its unique application as field emitter (Yi *et al.* 2007), logic gate (Ma *et al.* 2007) and CdTe has its application of being used in solar cells (Amin *et al.* 2007).

Our findings for cadmium telluride (CdTe) revealed that for various shapes the electronic behavior is also different. The electronic behavior reflected that for cadmium telluride the 2-atom linear and 2-atom zigzag wires are semi-conducting in nature while 4-atom square & 6-atom hexagonal shapes are insulating in nature (Kaushik *et al.* 2020). The study for cadmium Sulphide (CdS) nanowires by us established that 2-atom linear shape is conducting, 2-atom zigzag shape is semiconducting and 4-atom square shape & 6-atom hexagonal shapes are reflecting insulating characters (Singh et al. 2020). The ab-initio study for Zinc Selenide by us (Kaushik *et al.* 2020) for electronic properties reflected that the 2-atom zigzag wire is conducting, 2-atom linear wire is semiconducting whereas 4-atom square & 6-atom hexagonal shapes are coming out to be insulating here also. We (Kaushik *et al.* 2022) have also reviewed structural dependence for semiconducting material and the stability has also shown variation with

varying atomic arrangement. The electronic properties of Organic-Inorganic mixed halides-based perovskites have been analyzed by density functional theory is also being performed by us (Sharma *et al.* 2023) and found that addition of halogen shifts the energy bands in band structure which results in change of bandgap for of Solar Energy Material $CH_3NH_3PbX_3$ (X= I, Br and Cl) Perovskites. A model developed to study the effect of size and shape on the bandgap of semiconductor nanomaterials has found that bandgap increases by decreasing the size and depends on the shape considered (Paneru *et al*. 2023). The bandgap variation are reported (Singh *et al.* 2023) for spherical, thin film, nanowire, regular tetrahedral and regular octahedral shapes of semiconductor nanosolids. According to the study shape effect becomes prominent as the form changes from spherical to regular tetrahedral shape up to the size limit of 20 nm and concluded that the bandgap increases on decreasing size to the nanoscale.

2. Computational Details

The structures of the mentioned nanowires have been explored through Density Functional Theory calculations (Hohonberg *et al.* 1964; Kohn *et al.*1965). The pseudopotential technique has proven to be a very dynamic tool for studying electronic properties for different materials (Martin *et al.* 2009). Most of the above studies are performed by using ABINIT Code (Gonze *et al.* 2002).

3. Results

We have performed the extensive review with the available existing studies for electronic behavior for various semiconducting materials. The electronic properties show a drastic variation when the atomic arrangement is varied. The behavior of the semi conducting material under observation has shown all possible behavior. The detailed findings of the reviewed literature for electronic behavior are of critical importance.

The findings for electronic behavior for various materials as shown below in Table I reflect that 2-atom linear wire has come out to be conducting as well semiconducting for various materials depending on the nature of material, 2 atom zigzag wire has come out to be conducting for almost all materials other than cadmium sulphide and cadmium sulphide where it has shown semiconducting nature, 4-atom square shape has come out to be insulating for all materials other than gallium nitride and aluminum nitride where it has shown conducting nature but 6-atom hexagonal wire has come out to be insulating in all cases.

Table 1: Electronic Behavior Table for various Materials.

Material	Electronic Behavior of Material for Different Shapes			
	2-atom linear	2-atom zigzag	4-atom square	6-atom hexagonal
Gallium Nitride (GaN)	Semi-conducting	Conducting	Conducting	Insulating
Gallium Arsenide (GaAs)	Conducting	Conducting	Insulating	Insulating
Gallium Antimonide (GaSb)	Conducting	Conducting	Insulating	Insulating
Gallium Phosphide (GaP)	Conducting	Conducting	Insulating	Insulating
Aluminum Nitride (AlN)	Conducting	Conducting	Conducting	Insulating
Zinc Oxide (ZnO)	Semi-conducting	Conducting	Insulating	Insulating
Cadmium Sulphide (CdS)	Conducting	Semi-conducting	Insulating	Insulating
Zinc Selenide (ZnSe)	Semi-conducting	Conducting	Insulating	Insulating
Cadmium Telluride (CdTe)	Semi-conducting	Semi-conducting	Insulating	Insulating

4. Conclusion

The review on electronic properties of semiconducting nanowires has been performed. The structural dependence of electronic properties for different material by using ab initio DFT calculations have been analyzed. It is concluded that the electrical behavior of the nanowire is critically dependent on shape. A semiconducting nanowire may possess the conducting, semi conducting and insulating nature by varying the atomic arrangement i.e., the electronic behavior can be changed by changing the atomic arrangement for the materials under study. The present findings can be very carefully utilized while using these materials for designing various scientific devices.

Acknowledgements

We are also thankful to all researchers cited in the review. It is because of their reported work because of which we could perform the study and reach to such an important conclusion. We also express our gratitude for SCERT Gurugram, for providing infrastructural facilities because of which we could carry out our experimental work.

References

Abdalameer, N. K., S. N. Mazhir, and K. A. Aadim, 2020. The effect of ZnSe Core/shell on the properties of the window layer of the solar cell and its applications in solar energy. *Energy Reports*, *6*, 447-458.
DOI: 10.1016/j.egyr.2020.09.023

Amin, N., K. Sopian, and M. Konagai, 2007. Numerical modeling of CdS/CdTe and CdS/CdTe/ZnTe solar cells as a function of CdTe thickness. *Solar energy materials and solar cells*, *91*(13), 1202-1208.
DOI: 10.1016/j.solmat.2007.04.006

Chen, H. S., S. J. J. Wang, C. J. Lo, and J. Y. Chi, 2005. White-light emission from organics-capped ZnSe quantum dots and application in white-light-emitting diodes. *Applied Physics Letters*, *86*(13), 131905.
DOI: 10.1063/1.1886894

Fardi, H., and F. Buny, 2013. Characterization and modeling of CdS/CdTe heterojunction thin-film solar cell for high efficiency performance. *International Journal of Photoenergy*, *2013*.
DOI: 10.1155/2013/576952

Gonze, X., J. M. Beuken, R. Caracas, F. Detraux, M. Fuchs, G. M. Rignanese, and D. C. Allan, 2002. First-principles computation of material properties: the ABINIT software project. *Computational Materials Science*, *25*(3), 478-492.
DOI: 10.1016/S0927-0256(02)00325-7

Greytak, A. B., L. J. Lauhon, M. S. Gudiksen, and C. M. Lieber, 2004. Growth and transport properties of complementary germanium nanowire field-effect transistors. *Applied Physics Letters*, *84*(21), 4176-4178.
DOI:10.1063/1.1755846

Hohenberg, P., and W. Kohn, 1964. Inhomogeneous electron gas. *Physical review*, *136*(3B), B864.
DOI: 10.1103/PhysRev.136.B864

Kapadnis, R. S., S. B. Bansode, A. T. Supekar, P. K. Bhujbal, S. S. Kale, S. R. Jadkar, and H. M. Pathan, 2020. Cadmium telluride/cadmium sulfide thin films solar cells: a review. *ES Energy & Environment*, *10*(2), 3-12.

Kaushik, S. P., S. Singh, and R. K. Thakur, 2020. First Principles Study of Structural Stability and Electronic Properties of CdTe Nanowires. *Journal of Computational and Theoretical Nanoscience*, *17*(12), 5210-5214
DOI: 10.1166/jctn.2020.9631

Kaushik, S. P., R. K. Thakur, and S. Singh, 2022. Structural Dependence of Stability of Semiconducting Nanowires, *Shodhsankalp Journal*, 2(2), 25-31.

Kohn, W., and L. J. Sham, 1965. Self-consistent equations including exchange and correlation effects. *Physical review*, *140*(4A), A1133.
DOI: 10.1103/PhysRev.140.A1133

Li, D., Y. Wu, P. Kim, L. Shi, P. Yang, and A. Majumdar, 2003. Thermal conductivity of individual silicon nanowires. *Applied Physics Letters*, *83*(14), 2934-2936.
DOI: 10.1063/1.1616981

Lin, Y. F., Y. J. Hsu, S. Y. Lu, K. T. Chen, and T. Y. Tseng, 2007. Well-aligned ternary Cd1-x Zn x S nanowire arrays and their composition-dependent field emission properties. *The Journal of Physical Chemistry C*, *111*(36), 13418-13426.
DOI: 10.1021/jp074094b

Ma, R. M., L. Dai, H. B. Huo, W. J. Xu, and G. G. Qin, 2007. High-performance logic circuits constructed on single CdS nanowires. *Nano letters*, *7*(11), 3300-3304.
DOI: 10.1021/nl0715286
PMID:17935372

Martin, R. M., 2009. Electronic structure. *Physik Journal*, *8*(4), 54.

Monkhorst, H. J., and J. D. Pack, 1976. Special points for Brillouin-zone integrations. *Physical review*

B, *13*(12), 5188.
DOI: 10.1103/PhysRevB.13.5188

Nasieka, I., M. Boyko, V. Strelchuk, N. Kovalenko, A. Gerasimenko, N. Starzhinskiy, and D. Sofronov, 2014. Optical characterization of Er-doped ZnSe for scintillation applications. *Optical Materials*, *38*, 272-277.
DOI: 10.1016/j.optmat.2014.10.052

Paneru, P., and M. Kumar, 2023. Development of size and shape dependent model for bandgap of semiconductor nanomaterials. *Modern Physics Letters B*, 2350111.
DOI: 10.1142/S0217984923501117

Perdew, J. P., K. Burke, and M. Ernzerhof, 1996. Generalized gradient approximation made simple. *Physical review letters*, *77*(18), 3865.
DOI: 10.1103/PhysRevLett.77.3865
PMID:10062328

Prakash, K. S., S. Satyendra, and K. R. Krishna, 2020. Stability and electronic properties of ZnSe nanowires: An ab initio approach. Наносистемы: физика, химия, математика, *11*(5), 546-552.
DOI: 10.17586/2220-8054-2020-11-5-546-552

Sharma S., S. P. Kaushik, R. K. Thakur, and V. K. Shukla, 2023. Study of Structural Properties of Solar Energy Material $CH_3NH_3PbX_3$ (X= I, Br and Cl) Perovskites and its Mixed Halides Using Density Functional Theory, *Nepal Journal of Science and Technology*, 21(2).
DOI: 10.3126/njst.v21i2.62360

Singh, M., S. Lara, N. J. Matjelo, L. Lepekola, M. Kao, and M. T. Matobako, 2023. Modeling Size and Shape Dependence of Electro-Optical Properties of Semiconductor Nanosolids. *International Journal of Nanoscience*, 2350052.
DOI: 10.1142/S0219581X23500527

Singh, S., S. P. Kaushik, and Supreet, 2020. Ab Initio Study of Electronic Properties of Cadmium Sulphide Nanowires. *Journal of Computational and Theoretical Nanoscience*, *17*(2-3), 546-551.
DOI: 10.1166/jctn.2020.8907

Singh, S., P. Srivastava, and A. Mishra, 2009. Ab-initio study of gallium arsenide nanowires. *Journal of Computational and Theoretical Nanoscience*, *6*(7), 1556-1560.
DOI: 10.1166/jctn.2009.1211

Singh, S., P. Srivastava, and A. Mishra, 2009. Structural stability and electronic properties of GaSb nanowires. *Physica E: Low-dimensional Systems and Nanostructures*, *42*(1), 46-50.
DOI: 10.1016/j.physe.2009.08.017

Singh, S., and P. Srivastava, 2015. Ab-initio Study of the Structural Stability and Electronic Properties of ZnO Nanowires. In *2015 Fifth International Conference on Communication Systems and Network Technologies* (pp. 1279-1283), IEEE.
DOI: 10.1109/CSNT.2015.226
PMID:25711148

Srivastava, P., and S. Singh, 2011. Shape Dependent Electronic Properties of Aluminium Nitride Nanowires. *Journal of Computational and Theoretical Nanoscience*, *8*(9), 1764-1768.
DOI:10.1166/jctn.2011.1880

Srivastava, P., S. Singh, and A. Mishra, 2011. Electronic properties of GaP nanowires of different shapes. *Journal of Nanoscience and Nanotechnology*, *11*(12), 10464-10469
DOI: 10.1166/jnn.2011.3960
PMID:22408927

Srivastava, P., S. Singh, and A. Mishra, 2008. Stability and Electronic Properties of GaN Nanowires-An Ab-Initio Approach. *Journal of Computational and Theoretical Nanoscience*, *5*(4), 635-638.
DOI: 10.1166/jctn.2008.029

Tian, B., X. Zheng, T. J. Kempa, Y. Fang, N. Yu, G. Yu, and C. M. Lieber, 2007. Coaxial silicon nanowires as solar cells and nanoelectronics power sources. *Nature*, *449*(7164), 885-889.
DOI: 10.1038/nature06181
PMID:17943126

Troullier, N., and J. L. Martins, 1991. Efficient pseudopotentials for plane-wave calculations. *Physical review B*, *43*(3), 1993.
DOI: 10.1103/PhysRevB.43.1993
PMID:9997467

Vigue, F., E. Tournie, and J. P. Faurie, 2000. ZnSe-based Schottky barrier photodetectors. *Electronics Letters*, *36*(4), 352-354.
DOI: 10.1049/el:20000282

Effect of Starch Coating on Shelf-Life and Biochemical Properties of Carrot (*Daucus carota)*

Shanta Pokhrel[1*], Bibisha Basnet[1], Dhruba Raj Bhattarai[2], Anjali Shrestha[3], Anand Kumar Yadav[4], and Ishwori P. Gautam[3]

[1]Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Nepal
[2]Nepal Agricultural Research Council, Lalitpur, Nepal
[3]National Horticulture Research Centre, Nepal Agricultural Research Council, Lalitpur, Nepal
[4]Patan Multiple Campus, Patandhoka, Lalitpur, Nepal

***CORRESPONDING AUTHOR:**
Shanta Pokhrel
Email:shantabhattarai2014@gmail.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI : https://doi.org/10.3126/njst.v21i2.67169

Date of Submission: Nov 8, 2021

Date of Acceptance: Feb 12, 2022

ABSTRACT

A simple root vegetable (*Daucus carota*), often called as carrot, is usually conical or cylindrical in shape. It has a great medicinal value and different health benefits. The main objective of the study is to evaluate the effect of starch coating on shelf-life and biochemical properties of carrot. Total soluble solid (TSS) and titrable acidity (TA) was measured by hand refractometer method. The carrot treated with calcium chloride was more acidic 0.72 (±0.075) than distilled water 0.46 (±0.086) and starch 0.48 (±0.074) at 10 days after storage. Total soluble solid was found maximum at 10 days after storage (11.9 °Brix). As measured by spectrophotometer, carrot coated with starch had considerably high β-carotene content (21.78 mg/100 g) as compared to calcium chloride (17.39 mg/100 g) and distilled water (17.19 mg/100 g). Highest physiological weight loss was exhibited by calcium chloride as compared to distilled water and starch. Shelf-life of carrot was shorter in calcium chloride as compared to distilled water and starch; longest shelf-life was exhibited by starch coated carrot.

Keywords: β-carotene, physiological weight loss, shelf-life, titrable acidity, total soluble solid

1. Introduction

Carrot (*Daucus carota*) that belongs to family *Apiaceae* is a biennial herb grown for its edible root. It is an economically important horticultural crop that has become highly popular in recent decades due to increased awareness of its nutritional value (Arscott & Tanumihardjo 2010). Carrot is consumed fresh as well as cooked, either alone or with other vegetables, in the preparation of curries, soups, stews and pies. Fresh grated carrot is used in salad and tender roots are picked. Carrot root is healthy and highly nutritious because of enrichment with antioxidants, vitamins, dietary and mineral (Sharma *et al*. 2006; Mandal *et al*. 2017). It is also a good source of thiamine, vitamin A, and riboflavin (Thompson & Kelly 1957). Thus, it is associated with several health benefits. It is highly nutritious as it contains many important minerals, appreciable amounts of vitamins

B1, B2, B6, B12 and it is rich in β-carotene. β-carotene (Fig. 1) is a precursor of vitamin A and is reported to prevent cancer (Ong *et al* 1983). Carotenoids give yellow, orange, and red colors of the carrots, whereas anthocyanins (polyphenolic compounds) provide the purple color to carrots (Arscott & Tanumihardjo 2010). These pigments are health beneficial and provide protection from certain types of cancer and cardiovascular diseases (Semwal *et al*. 2016) and consumer interest in natural whole foods rich in these compounds, often referred to as functional foods, is growing (Hasler & Brown 2009). Carotenoids are a group of phytochemicals that comprises a family of over 700 compounds in nature and are responsible for the pigmentations in many fruits and vegetables (Britton *et al*. 2004). Carrots roots are rich in carotenoids which can be routinely separated and quantified in typical and dark orange carrots. The dominant forms of carotenoids are the provitamin A and carotenes (α and β-carotene) accounting for 13 to 40% and 45 to 80% of the carotenoids in orange carrots respectively (Simon & Wolff 1987). Several studies have reported that ripening can be slow down, color changes can be delayed, water loss and decay can be decrease and appearance can be improved by applying edible coating technology which is a simple and environmentally friendly technique (Park *et al*. 1994 (a,b); Baldwin, 2001; Rashidi *et al.* 2009). Edible coatings can be performed by using polysaccharides, proteins, lipids or a blend of these compounds (Rashidi *et al*., 2009). Their presence and abundance create the barrier properties of material such as water vapor, oxygen, carbon dioxide and lipid transfer in food systems (Guilbert *et al*. 1996). Starch and chitosan prolong storage life and control the decay of fruits by reducing the growth of many phytopathogenic bacteria and fungi because of its semi permeable film forming ability and biochemical properties (Jiang *et al*. 2001; Pokhrel *et al*. 2021). Edible films of starch are colorless, odorless, tasteless, nontoxic and biodegradable. They show very low permeability to oxygen at low relative humidity (Phan The *et al*. 2009) and are suggested for their uses in food product protection to improve quality and shelf life (Flores *et al*. 2007; Pokhrel 2015). This research was conducted to identify the effect of starch coating on shelf life and biochemical properties of Nepalese carrot.

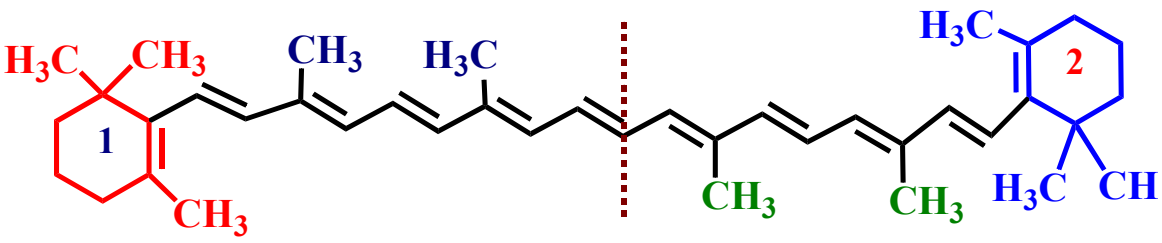


Fig. 1: Structure of β-carotene

2. Methodology

2.1 Plant Materials

Samples were brought from Mulpani Agricultural Organic Farm Kathmandu, Nepal. The cultivation conditions were same for all the species. The samples were washed in the cold water, sliced and stored for further analysis. Acetone (Parsol Chemical Pvt. Ltd. Mumbai, India), petroleum ether (B. Joshi Agrochem Pharma Mumbai, India), sodium sulphate (Lakshita Chemicals, Mumbai, India), β-carotene (Divis Nutraceuticals, Hyderabad, India), methanolic KOH (Paramount Acid and Chemicals Corporation, Mumbai, India), distilled water (Ultra Super TM, Marech Pvt. Ltd. Lalitpur, Nepal), calcium chloride (Shiv Shakti Chloride and Chemicals, Gujrat, India), and starch (Surya Agro Products Pvt. Ltd., Nepal)were provided by the National Horticulture Research Centre, Nepal Agricultural Research Council, Lalitpur, Nepal. Hand refractometer (Atago CO. Ltd.), and spectrophotometer and double-distilled water were used throughout the analysis.

2.2 Methodology

2.2.1 Titrable acidity

The samples paste was taken in a volumetric flask and its weight was measured. The volume was made up to 100 mL with distilled water. The sample was cut into small pieces and was taken in a volumetric flask. The sample fruit was weighed. About 20 g of the sample

was put into the mixture and grinded. The juice was extracted with the help of cotton. The solution was filtered and 5mL of the sample wastaken in a beaker. The volume was made up to 100 mL with distilled water. The refractometer was cleaned with the help of distilled water and a few drops of sample juice were placed on the refractometer. The cover plate was lowered and the reading was noted. Finally, acidity of the sample was calculated (KC & Rai 2012).

2.2.2 Total Soluble Solid

The sample was taken in a volumetric flask and its weight was measured and the sample (20 g) was grinded. The juice was extracted with the help of cotton. Few drops of sample juice were placed in the refractometer. The cover plate was lowered and the reading was noted (KC & Rai 2012).

2.2.3 β-Carotene Extraction

First of all, 5 g sample and 62.5 mL acetone were taken in a mixture. The solution was blended/rotated for 5 min. Then the solution was filtered with the help of funnel. It was washed by acetone until the solution was colorless. The second step was separation. The filtrate solution was passed to the separatory funnel. 25 mL of petroleum ether and 10 mL of sodium sulphate (10%) were added in a separatory funnel. The funnel was shaken well, the lower phase was discarded to next separatory funnel to get the residual amount of β-carotene as the lower phase. 25 mL petroleum ether were added to the second funnel then it was shaken and lower phase was discarded. The upper layer was pooled with ether extract in the separatory funnel. Then 12.5 mL of acetone were added in it. The funnel was shaken well and lower phase was discarded. Again, 12.5 mL of methanolic KOH were added in the separatory funnel and shaken well. The lower layer was discarded and 80 mL of water were added in it. Then, the solution was shaken well and the lower layer was discarded. The extract solution was filtered through the Whatmann filter paper 1 and the volume was made up to 100 mL with petroleum ether in a volumetric flask. The absorbance of the sample was measured at 450 nm against β-carotene standard. For the standard solution, β-carotene (5 mg) was dissolved in 0.5 mL chloroform and the volume was made up to 50 mL by petroleum ether to give standard concentration of 0.0001 g/mL (or 0.1 mg/mL or 100 µg/mL). The standard concentration versus absorbance was plotted in the graph for the determination of slope value (KC & Rai 2012). Using the value obtained from the curve, the carotene in the sample was calculated by using equation (1),

$$\text{Carotene } (\mu g/g) = \frac{\text{concentration from the curve} \quad \text{final volume (mL)} \quad \text{dilution}}{\text{weight of sample g}} \qquad (1)$$

2.2.4 Physiological Weight Loss (%)

Eighteen carrots in each treatment were labeled and the weight of each carrot was recorded with electronic digital balance in alternate day and mean was calculated (KC & Rai 2012). Physiological weight loss was determined by using equation (2).

$$\text{Weight loss } (\%) = \frac{\text{Initial weight} \quad \text{Final weight}}{\text{Initial weight}} \quad 100 \qquad (2)$$

2.2.5 Shelf Life

Shelf life was determined up to 50% acceptability of carrot (KC & Rai 2012).

3. Results and Discussion

3.1 Titrable Acidity (TA)

Fig. 2 shows that the titrable acidity of carrot increases during storage. Table 1 shows details about total acidity among the three treatments of carrot. Titratable acidity is directly related to the concentration of organic acids present in the carrot (Ghasemnezhad *et al*. 2010). TA of distilled water ranged from 0.35 (±0.0354) to 0.46 (±0.086). Similarly, that of calcium chloride ranged from 0.22 (± 0.0009) to 0.72 (± 0.075) and starch from 0.18 (± 0.013) to 0.48 (± 0.074) at various days after storage. From this result it was found that carrot treated with calcium chloride was more acidic followed by the carrot treated with starch and distilled water at 10 days after storage. However, Mandal *et al*. (2017) reported that chitosan (2%) coated with carrot remained with higher titrable acidity 0.22 at 5 days after storage (DAS) and 0.45 at 10 DAS.

Table 1: Titrable acidity of carrot at various days after storage (DAS) as affected by distilled water, calcium

chloride, and starch treatment.

Treatments	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS
Distilled Water	0.35 (±0.0354)	0.25 (±0.00016)	0.19 (±0.0002)	0.35 (±0.018)	0.46 (±0.086)
Starch	0.18 (±0.013)	0.26 (±0.0002)	0.27 (±0.007)	0.47 (±0.046)	0.48 (±0.074)
Calcium Chloride	0.22 (±0.0009)	0.36 (±0.037)	0.33 (±0.021)	0.4 (±0.046)	0.72 (±0.075)

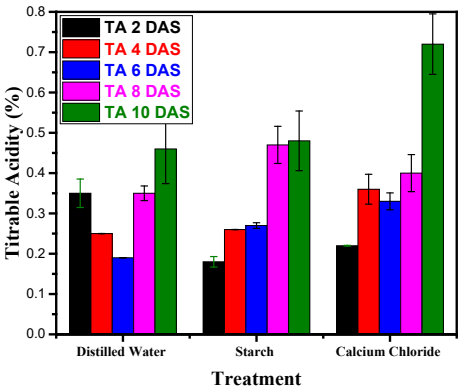


Fig. 2: Titrable acidity of carrot at various days after storage (DAS) as affected by distilled water, calcium chloride, and starch treatment.

3.2 Total Soluble Solid (TSS)

A significant variation in total soluble solid (TSS) content was noted in different treatments of carrot (Table 2). Carrot treated with calcium chloride showed TSS value (3.36, 3.23, 5.93, and 8.16 and 11.90 °Brix) at 2, 4, 6, 8 and 10 DAS respectively. Total soluble content (TSS) was found to increase with the storage time and its highest value was found 11.90 °Brix in carrot treated with calcium chloride after 10 days. These results are in agreement with the results reported by Niari *et al*. (2013) and Rashidi *et al*. (2009) that TSS was significantly increased by increasing storage period with edible coatings.

Table 2: Total soluble solid (TSS) of carrot at various days after storage (DAS) as affected by distilled water, calcium chloride and starch treatment.

Treatments	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS
Distilled water	7.16 (±0.094)	6.63 (0.126)	9.86 (±0.044)	8.43 (±0.094)	9.06 (±0.368)
Starch	4.20 (±0.077)	6.83 (±0.170)	7.73 (±0.094)	11.23 (±0.593)	9.90 (±0.293)
Calcium chloride	3.36 (±0.043)	3.23 (±0.044)	5.93 (±0.046)	8.16 (±0.262)	11.90 (±0.354)

3.3 β-Carotene

The absorption peaks shown by the carrot extract (see Table 3 for absorption peak values) as measured in absorption spectrophotometer were found to occur at the same wavelength with a maximum absorption peak (λ_{max}) at 450 nm as in absorption curve of β-carotene reported by John Scott 2001 (Karnjanawipagul *et al*. 2010; Suryana *et al*. 2013). Therefore, the sample extracted from carrot was valid as β-carotene. There was a marked decrease in the β-carotene content of the treatment at various DAS. The decrease in carotenoids is possibly due to the reduced ethylene emission rates and slows down the ripening process for the Aloe-coated tomato fruits (Chrysargyris *et al*. 2016). It was observed that at 4 DAS, it was ranged between 339.7 and 279.45 mg/100 g where as it ranged between 150.44 and 174.14 mg/100 g at 8 DAS and further reduced and ranged between 17.19 and 21.78 mg/100 g at 12 DAS which can be illustrated in Table 3. Carrot treated with starch had considerably high β-carotene content (21.78 mg/100 g) at 12 DAS, compared to other treatments. Similarly, Mandal *et al.* (2017) reported that carrot coated with chitosan 2% and carboxymethyl cellulose 2% had considerably high β-carotene content during the storage period.

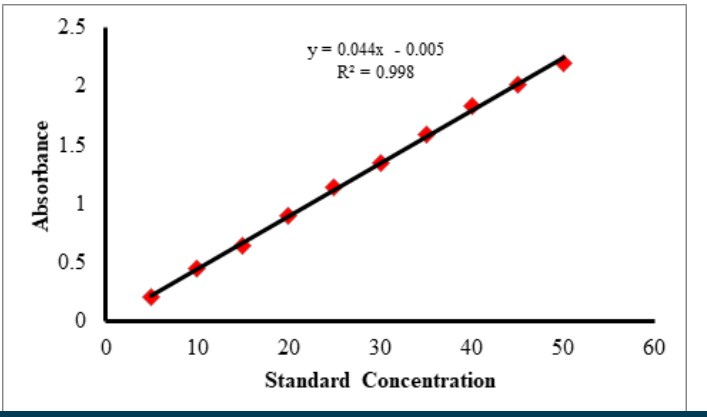


Fig. 3: Absorbance of the sample against β-carotene standard at 4 days after storage

Table 3: Determination of β-carotene from solvent partition method.

4 Days after storage				
Treatments	**Wt. of sample (g)**	**Absorbance (450 nm)**	**Concentration (y= 0.0055x-0.0167)**	**Amount (mg/100 gm)**
Distilled water	5	0.9175	169.8545455	339.709091
Calcium chloride	5	0.8003	148.5454545	297.090909
Starch	5	0.7518	139.7272727	279.454545
8 Days after storage				
Treatments	**Wt. of sample (g)**	**Absorbance (450 nm)**	**Concentration (y= 0.0055x-0.0167)**	**Amount (mg/100 gm)**
Distilled water	5	0.6497	75.22352941	150.447059
Calcium chloride	5	0.7298	84.64705882	169.294118
Starch	5	0.7504	87.07058824	174.141176
12Days after storage				
Treatments	**Wt. of sample (g)**	**Absorbance (450 nm)**	**Concentration (y= 0.0055x-0.0167)**	**Amount (mg/100 gm)**
Distilled water	5	0.4525	8.599534342	17.1990687
Calcium chloride	5	0.4611	8.699630757	17.3993015
Starch	5	0.6493	10.89057043	21.7811409

3.4 Physiological Weight Loss

The physiological weight loss of carrots treated with calcium chloride, starch and distilled water determined at alternate days (Fig. 4) and the dehydration condition of the carrot samples were the same as all the samples taken were fresh cut. At 2 days of storage, the weight loss of carrot samples treated with distilled water, starch and calcium chloride was 2.16 (±0.18), 2.26 (±0.30) and 1.79 (±0.15) percentage respectively. As compared to 2 DAS and 14 DAS the weight loss of calcium chloride treated carrot was increased from 1.79 (±0.15) to 2.83 (±0.16). However, the weight loss of carrot treated with distilled water was decreased from 2.16 (±0.18) to 1.29 (±0.06) and that of starch coated carrot decreased from 2.62 (±0.30) to 1.24 (±0.10). From the result, it was found that the highest loss was exhibited by carrot treated with calcium chloridefollowed by the carrot sample treated with distilled water and the least weight loss was shown by starch coated samples. Arnon *et al*. (2014) reported that carrots treated with chitosan and carboxymethyl cellulose had low physiological weight loss during storage.

3.5 Shelf-Life

For shelf-life study carrots were kept until 50% acceptability. Among the three treatments, the shelf-life was the shortest in the carrot sample coated with calcium chloride (17 days) followed by the sample coated with distilled water (23 days) and the longest shelf-life was shown by starch coated carrots (27 days). The longest shelf-life in starch was associated with bigger fruit size, a greater number of locules per fruit and lower weight loss. However, Mandal *et al.* (2017) reported that carrot treated with chitosan 2% and carboxymethyl cellulose had maximum shelf-life (25.5 days) followed by treatment with chitosan 1% and carboxymethyl cellulose (shelf-life 21.25 days) and chitosan 2% (shelf-life 20.25) days.

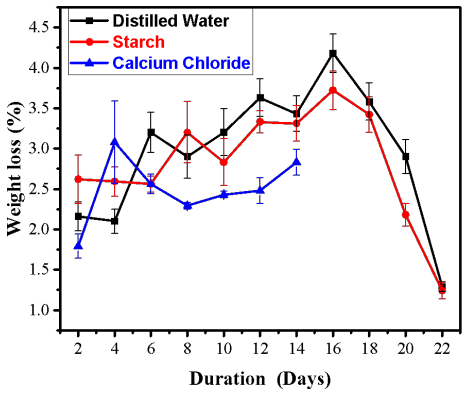


Fig. 4: Physiological weight loss (%) of carrots at various days after storage

4. Conclusion

On the basis of above findings, it can be concluded that there is increase in the titrable acidity (TA) and total soluble solid (TSS) with the storage time as observed in calcium chloride treated carrot at 10 DAS, whereas the β-carotene value was found to decrease with the storage time in all as observed in all carrots treated with distilled water, starch and calcium chloride. There was not much difference in TSS and titrable acidity among the treatments. β-carotene content was found to decrease with the storage duration of the carrots whereas highest physiological weight loss was exhibited by calcium chloride treated carrot followed by the samples treated with distilled water and the lowest decrease in β-carotene content was shown by starch coated carrot. Shelf-life was found longest viz. 27 days by starch coated carrots. Thus, this study reveals that starch coating is highly effective for increasing the postharvest shelf-life of carrot.

The results exclude the quantitative analysis of the thickness of coating; it has to be further confirmed by means of analytical and gravimetric tools. The β carotene content in various DAS could be affected by atmospheric humidity, temperature, method of carotene extraction etc. The optimized conditions and sources of experimental errors have not been studied; and this is the limitation of the research, and can be quantified by a future perspective study.

Acknowledgements

We are highly thankful to University Grant Commission (UGC), Sanothimi, Bhaktapur, Nepal for providing research funds (SRDIG-79/80-01) for First Author (S.P.). The authors are grateful to Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal for providing laboratory facilities to conduct experimental work. National Horticulture Research Centre, Nepal Agricultural Research Council (NARC), Lalitpur, Nepal is also gratifying for the research collaboration.

References

Arnon, H., Y. Zaitsev, R. Porat, and E. Poverenov, 2014. Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus fruit, Journal of Postharvest Biology and Technology 87:21-26
DOI: 10.1016/j.postharvbio.2013.08.007

Arscott, S. A., and S. A. Tanumihardjo, 2010. Carrots of many colors provide basic nutrition and bioavailable phytochemicals acting as a functional food. Comprehensive Reviews in Food Science and Food Safety 9(2):223-239.
DOI:10.1111/j.1541-4337.2009.00103.x

Britton, G., S. Liaaen-Jenson, and H. A. Pfander, 2004. Carotenoids Handbook. Journal of Switzerland 134:1081-1083.
DOI: 10.1007/978-3-0348-7836-4

Chrysargyris, A., A. Nikou, and N. Tzortzakis, 2016. Effectiveness of Aloe vera gel coating for maintaining tomato fruit quality, New Zealand Journal of Crop and Horticultural Science, 44(3):203-217.

DOI: 10.1080/01140671.2016.1181661

Flores, S., A. S. Haedo, C. Campos, and L. Gerschenson, 2007. Antimicrobial performance of potassium sorbate supported in tabioca starch edible films. European Food Research Technology 225:375-384.
DOI: 10.1007/s00217-006-0427-5

Ghasemnezhad, M., M. Shiri, and M. Sanavi, 2010. Effect of chitosan coatings on some quality indices of apricot (*Prunus armeniaca* L.) during cold storage. Caspian Journal of Environmental Sciences 8(1):25-33.

Guilbert, S., N. Gontard, and L. G. M. Gorris, 1996. Prolongation of the shelf-life of perishable food products using biodegradable films and coatings. Lebensmittel-Wissenschaft & Technologie 29:10-17.
DOI: 10.1006/fstl.1996.0002

Hasler, C. M., and A. C. Brown, 2009. Position of the functional foods.Journal of American Dietetic Association109:735-746.
DOI: 10.1016/j.jada.2009.02.023 PMID:19338113

Jiang, Y., and Y. Li, 2001. Effects of chitosan coating on postharvest life and quality of longan fruit. Food Chemistry 73:139-143.
DOI: 10.1016/S0308-8146(00)00246-6

KC, B. J., and B. K. Rai, 2012. Determination of β-carotene by solvent partition method. Journal of Basic Food Analysis Handbook.

Mandal, D., V. Ngohla, Veronica, T. Hazarika Kumar, and A. Shukla Chandra, 2017. Influence of 6-Benzylaminopurine, chitosan and carboxy methyl cellulose on quality and shelf life of fresh cut carrot (*Daucus D. carota*) shreds under refrigerated storage. International Journal of Bio-research and Stress Management 8:69-74.
DOI: 10.23910/IJBSM/2017.8.1.1739b

Nairi, S. M., M. H. Bahrt, and M. Rashidi, 2013. Effects of coating methods and storage periods on some quality characteristics of carrots during ambient storage. Journal of World Applied Science 21:1025-1031.

Ong, D. E., F. Chytil, and E. D. Aurbach, 1983. Vitamins and hormones. Journal of New York Academic Press 13:105-112.
DOI: 10.1016/S0083-6729(08)60433-1
PMID:6324478

Phan The, D., F. Debeaufort, A. Voilley, and D. Luu, 2009. Biopolymer interactions affect the functional properties of edible films based on agar, cassava starch and arabinoxylan blends. Journal of Food Engineering 90:548-558. DOI: 10.1016/j.jfoodeng.2008.07.023

Park, H. J., M. S. Chinnan, and R. L. Shewfelt, 1994a. Edible coating effects on storage life and quality of tomatoes. Journal of Food Science 59:568-570.
DOI: 10.1111/j.1365-2621.1994.tb05563.x

Park, H. J., M. S. Chinnan, and R. L. Shewfelt, 1994b. Edible corn-zein film coatings to extend storage life of tomatoes. Journal of Food Processing and Preservation 18:317-331.
DOI:10.1111/j.1745-4549.1994.tb00255.x

Baldwin, E. A., 2001. New coating formulations for the conservation of tropical fruits, http://technofruits2001.cirad.fr 10/08/2002.

Karnjanawipagul, P., W. Nittayanuntawech, P. Rojsanga, and L. Suntornsuk, 2010. Analysis of β-carotene in carrot by spectrophotometry, Mahidol University Journal of Pharmaceutical Sciences 37:8-16.

Pokhrel, S., 2015. A review on introduction and applications of starch and its biodegradable polymers. International Journal of Environment 4(4):114-125.DOI: 10.3126/ije.v4i4.14108

Pokhrel, S., S. Shah, and H. S. Adhikari, 2021. Synthesis and characterization of chitosan from prawn shells and study of its effects on weight loss of *Myrica esculenta* fruits. Asian Journal of Chemistry 33(2):299-306.
DOI: 10.14233/ajchem.2021.22980

Rashidi, M., M. H. Bahri, and S. Abbassi, 2009. Effects of relative humidity, coating methods and storage periods on some qualitative characteristics of carrot during cold storage. American-Eurasian Journal of Agricultural & Environmental 5:359-367.

Semwal, S., N. Chaudhary, and S. Karoulia, 2016. Addition of carrot pomace to increase the nutritional and rheological properties of traditional cake. International Journal of Scientific Research 5:1412-1416.DOI: 10.21275/v5i5.NOV163676

Sharma, H. K., J. Kaur, B. C. Singh, and A. A. Shilandi, 2006. Optimization of pretreatment conditions of carrots to maximize juice recovery by response surface methodology.Journal of Engineering Science and Technology 1:158-165.

Simon, P. W., and X. Y. Wolff, 1987. Carotene in typical and dark orange carrots. Journal of Agriculture and Food Chemistry 35:1077-1122.
DOI: 10.1021/jf00078a038

Scott, K. J., 2001. Detection and measurement of carotenoids by UV/VIS spectrophotometry. Current Protocols in Food Analytical Chemistry, 1:F2-2.
DOI: 10.1002/0471142913.faf0202s00

Suryana, R., Khoiruddin, and A. Supriyanto, 2013. Beta-carotene Dye of *Daucus carota* as Sensitizer on Dye-Sensitized Solar Cell, Materials Science Forum 737, 15-19.
DOI: 10.4028/www.scientific.net/MSF.737.15

Thompson, H. C., and W. C. Kelly, 1957. Vegetable crops edition. Journal of Macgrow Hill Book 65:405-430.

Assessing the Climate Change Vulnerability of the Communities Residing in Doda River Basin, Far Western Nepal

Tark Raj Joshi[1], Satyam Kumar Chaudhari[2], Prakriti Koirala[2], Mahesh Prasad Awasthi[1], Sudeep Thakuri[2,3], Ramesh Raj Pant[2], Kumari Tulshi Bhatta[2] and Pawan Kumar Neupane[4]

[1]Faculty of Science and Technology, Far Western University, Mahendranagar, Kanchanpur, Nepal.
[2]Central Department of Environmental Science, Tribhuvan University, Kirtipur, Kathmandu, Nepal.
[3]Graduate School of Science and Technology, Mid-West University, Birendranagar, Surkhet, Nepal.
[4]Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal.

***CORRESPONDING AUTHOR:**
Tark Raj Joshi
Email:tarkraj2001@gmail.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI :
https://doi.org/10.3126/njst.v21i2.67170

Date of Submission: Jan 14, 2024

Date of Acceptance: May 1, 2024

ABSTRACT

The study evaluates the vulnerability and climate change (CC) impacts on livelihood-related services aiming to identify strategies for enhancing resilience and adaptation. Key aspects of the study include the analysis of hydro-meteorological data, examination of climatic variability evidence, and vulnerability assessments related to CC. Vulnerability to CC varies based on exposure, sensitivity, and adaptation capacity, especially within a small spatial scale. Employing a bottom-up approach, the study applied trend analysis, Mann-Kendall statistical trend, IPCC vulnerability equation, and the Capacity Building and Vulnerability Assessment (CBCA) framework as fundamental methodologies. Meteorological and household data validate climatic trends and vulnerability. Significant changes in climatic parameters are observed, mirroring previous studies. Local communities experience decreased rainfall frequency, frequent floods, extended dry seasons, delayed monsoons, and intense late summer rainfall, verified by data from the nearest meteorological station. The exposure index ranges from 2.35 to 3.87, with wards 1 and 6 of Laljhadi Rural Municipality (RM) having the least exposure while ward 3 the highest, according to respondent perceptions. Sensitivity is highest in wards near the Doda River, with ward 4 having the highest sensitivity index. Adaptive capacity indices range from 2.01 to 3.68, with the least in wards 1 and 6. The highest vulnerability is observed in wards 3, 4, and 2 with vulnerability scores of 16.8, 13.6, and 11.1, respectively. Overall, the vulnerability calibration index indicated low adaptive capacity across all wards, emphasizing the need to enhance adaptive capabilities as a key recommendation for reducing CC vulnerability.

Keywords: Climate change, Trend analysis, Vulnerability, Adaptation strategies

1. Introduction

It's unequivocal that the global climate change is raising threats to the earth's system and human wellbeing. The impacts mainly threaten the countryside communities that are more dependent on natural resources (Maharjan *et al*. 2011). While climate change poses a universal challenge, its repercussions vary across regions, nations, industries, and communities. The scientific and political communities are increasingly prioritizing the examination of climate change and its effects on livelihoods. Projections indicate that the consequences of climate change will exert significant and detrimental impacts on both societies and economies (Panthi *et al*. 2016; Poudel *et al*. 2020; Sujakhu *et al*. 2019). The vulnerability to climate change is contingent upon diverse factors and varies across locations, sectors, and communities. Those residing in developing nations, relying predominantly on subsistence livelihoods linked to agriculture and livestock production, are notably recognized as vulnerable. Nepal, characterized by a predominant mixed agro-livestock system among its population, has been identified as the fourth most vulnerable country to climate change globally (Panthi *et al*. 2016).

People who are socially, economically, culturally, politically, institutionally or otherwise marginalized are vulnerable to CC (IPCC 2014) because adaptive capacity to CC depends on physical resources, access to technology and information, varieties of infrastructure, institutional capability, and the distribution of resources (Yohe & Tole 2002). The degree of a system's sensitivity to climatic hazards also depends on socio-economic factors such as population and infrastructure (Parry *et al*. 2005). Climate vulnerability, the extent of a system or a community being susceptible to or unable to cope with the adverse effects of CC including climate variability and extremes, is an urgent issue among many countries, impoverished and developing ones (Giri *et al*. 2021). It is a function of exposure, sensitivity, and adaptive capacity regarding a specific risk (IPCC 2014) and depends not only on the system's sensitivity but also its ability to adapt to new climatic conditions. (Cutter *et al*. 2000) points out that vulnerability to CC can be decayed into three distinct components; risk of exposure to hazards, capability for social response, and attribute of places such as geographical location.

The concept of CC vulnerability helps to understand the cause/effect relationship behind CC, its impact on people, the economy, and socio-ecological systems (Fritzsche *et al*. 2014). On the other hand, Vulnerability Assessments (VAs) and Climate Risk Assessments (CRAs) help identify the nature and extent to which CC and its impacts may harm a country, region, sector, or community. The assessments of vulnerability and/or climate risks are therefore a central component of adaptation action. Vulnerability varies across temporal and spatial scales (Kayastha *et al*. 2023) and depends on economic, public, geographic, cultural, institutional, and environmental factors (Giri *et al*. 2021; Kayastha *et al*. 2023; Pandey & Jha 2012). In many developing countries, including Nepal, high vulnerability and direct exposure are also the outcomes of shortsighted development processes made more inadequate by environmental mismanagement (Dixit *et al*. 2015). Nepal, with predominantly natural resource-based livelihoods, and a low level of adaptive capacity due to higher incidence of poverty, is placed among the most vulnerable country to CC (Oxfam 2009).

The latest study shows that the maximum temperature of Nepal increased by 0.45 °C per decade from 1976–2015 (Thakuri *et al*. 2019). It is predicted that intense precipitation of quick duration will intensify problems like flooding, landslides, and sedimentation (NCVST 2009; NAPA 2010). Vulnerability science operates on the premise that human populations play a mediating role in environmental change, thereby influencing its impacts. Consequently, the bottom-up approach can be applied in crucial policy and practical contexts, emphasizing the interconnectedness of society and nature (Pandey & Jha 2012). There is a scarcity of knowledge and information regarding the specific ways in which climate hazards are affecting livelihood resources, the diverse impacts of climate change on various well-being groups, and the responses of impoverished individuals in the mountainous regions of Nepal to climate changes. Examining the varied impacts of climate change is crucial in Nepal's historically hierarchical society, characterized by widespread poverty linked to rural-urban disparities, geographical variations, gender distinctions, and caste/ethnic divisions (Aryal *et al*. 2014; Bista 2023; Gentle & Maraseni 2012)adaptive capacity is low due to limited information, poor access to services, and inequitable access to productive assets. Few studies have reported on the current status of rural and remote mountain areas in Nepal with little known about adaptation strategies in use. This article is based on a study in the remote mountainous Jumla District of Nepal to explore how

climate change is affecting the livelihood of local communities and how different wellbeing groups are differentially impacted. Looking from a wellbeing lens, adaptation practices by households as well as local support mechanisms were explored to predict the severity of effects now and into the future. Using a climate vulnerability and capacity analysis (CVCA.

The present study analyzed the perceptions and evidence of climate variability, adaptive capacity, and vulnerability to CC among rural communities in the Doda River Basin, Laljhadi RM, Kanchanpur district, Sudurpashchim Province, Nepal. Utilizing a bottom-up assessment approach, the research explores local perspectives on climatic and non-climatic drivers of change and their effects on livelihood-related services. The goal is to identify adaptation options for creating a climate-resilient community. The study addresses academic and policy needs by developing a vulnerability index to assess climate change vulnerability at the community level. The methodology aims to connect community priorities with macro-level policies, emphasizing that higher-level planning should be informed by insights gained at the local level. Through a bottom-up approach, the proposed index identifies urgent adaptation needs and local coping strategies, providing recommendations for prioritized action and contributing to a comprehensive, integrated model for enhancing climate resilience at national, regional and local levels.

2. Materials and Methods

2.1 Study Area

Doda River Basin, spanning from 28°50'59.69" N to 28°39'54.68" N and 80°20'15.9" E to 80°30'0.54" E, encompasses an area of 154.65 km^2. It includes two primary river channels, the Syaali River and the Banara River, situated within Laljhadi Rural Municipality. It is located in Laljhadi RM, with predominantly flat topography and is positioned 46 km to the east of the district headquarters, Bhimdatta Nagar, in the Kanchanpur district of the Sudurpashchim Province, Nepal (Fig. 1). The population of the Rural Municipality is reported as 25,037 according to the census conducted in 2078 BS. The study zone primarily covers the six wards of Laljhadi RM. Krishnapur and Punarwas Municipality lie to the east of Laljhadi RM, with Shuklaphanta and Belauri Municipality comprising its western boundaries. The northern boundaries are shared with Shuklaphanta and Krishnapur Municipality, while to the south, it is contiguous with Belauri and Punarwas Municipality. Laljhadi RM holds significant religious, social importance, and possesses unique characteristics. The predominant ethnic groups in Laljhadi Rural Municipality are Tharu, Bahun/Chetri, and Dalit, with RanaTharu also considering this area as their home. The RanaTharu community constitutes approximately 80% of the local population. Kanchanpur district is classified as moderately vulnerable concerning temperature, rainfall, and flood vulnerability (MoE 2010).

The study area is characterized by a subtropical climate, typical of the Terai region of Nepal. The wet season typically spans from June to September, coinciding with the South Asian monsoon, during which the area receives the majority of its precipitation. The average annual rainfall over the 34-year span from 1985 to 2019 is about 3459.79 mm, however average annual rainfall in winter 142.68 mm, pre-monsoon 179.61 mm, monsoon 2468.75 mm and post monsoon 99.92 mm. The study area accounts an average maximum temperature of 30.55°C and an average minimum temperature of 17.58°C for the same 34-year period, as documented by weather stations situated in Attariya (station no. 209) and Mahendranagar (station no.105). In terms of land use, agricultural land occupies approximately 6654.85 ha (43.17%), water bodies encompass about 1153.43 ha (7.48%), settlement areas encompass around 1026.13 ha (6.66%), and forest area spans about 6579.82 ha (42.69%).

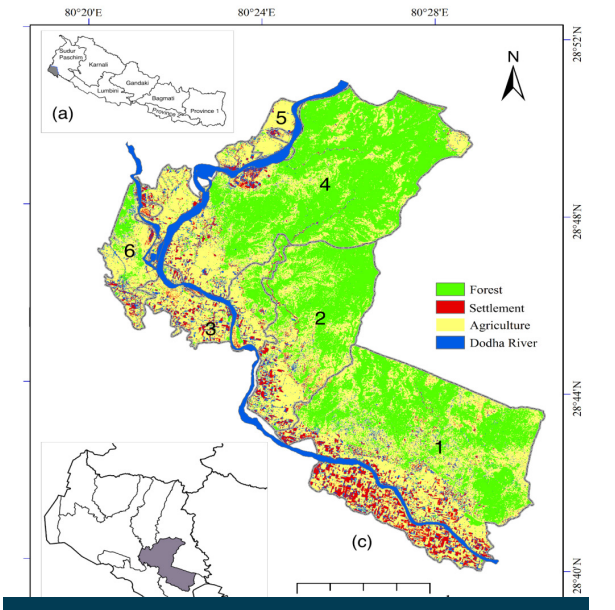


Fig. 1: Map showing the study area with land cover classification in Laljhadi RM, Kanchanpur district, Sudurpachim Province (b). A map of Nepal is shown in the inset (a).

2.2 Analytical Procedures and Methods

An analytical framework was developed for a systematic assessment of the factors contributing to vulnerability. Subsequently, a comprehensive approach to data collection was executed, incorporating both quantitative and qualitative methods. The objective of this methodology was to thoroughly appraise the vulnerability of the specified study site by employing the parameters established in the framework. Table 1 outlines the parameters and indicators used to evaluate the components of vulnerability, categorized into Adaptive Capacity, Exposure, and Sensitivity. For each criterion, specific indicators were identified and rated on a scale of 1 to 5, ranging from very low to very high perception, as per the NAPA (GON), 2010, and household questionnaire sources.

Table 1: Parameters and indicators used for assessing the vulnerability components

Parameter	Indicator
Adaptive Capacity	
Human	Literacy rate, Per capita income, Awareness on CC, Profession
Social	Households affiliated to a formal and informal institution, Number of CBOs, NGOs, GOs,
Financial	Total number of cooperatives and banks
Physical	Infrastructure for services, schools, metaled road, bridge
Information	Communication source
Exposure	
Temperature	Hot days, hot waves, and cold waves
Precipitation	Monsoon Rainfall, winter rainfall, change in rainfall duration
Indicator	Appearance and disappearance of species, change in sowing period
Climate-induced disaster	Flood and drought events
Sensitivity	
Agriculture and food security	Loss of crop production, livestock products, agricultural land, a decline in cash crop productivity
Forest and biodiversity	Forest coverage, increasing and spreading of invasive species, forest fodder dependency, a decline in forest products
Human settlement and infrastructure	Community settlement and Infrastructure, types of the shed, distance from the river
Water resources	Water bodies and their quality
Energy Demand	Dependency on traditional biomass energy
Gender	Total number of male and female ratio

**Each criteria indicator was ranked as 1 for very low, 2 for low, 3 for medium, 4 for high, and 5 for very high perception (Source: NAPA 2010 & HH Questionnaire)*

2.3 Data Collection

A comprehensive field survey was carried out at the study site, encompassing household surveys, key informant interviews, focus group discussions, and community transect walks to gather primary data. Climatic data, specifically temperature and rainfall information, were acquired from two meteorological stations: Station Index 209 in Attariya and Station Index 105 in Mahendranagar. These data, spanning 35 years from 1985 to 2019, were obtained from the Department of Hydrology and Meteorology, Government of Nepal (GON/DHM 2020), with a monthly temporal resolution.

2.3.1 Household Survey

A systematic approach was employed to survey 145 households out of a total of 3909 households in Laljhadi Rural Municipality, chosen through random sampling, to obtain the representative necessary data. A combination of quantitative and qualitative data from the community was gathered through interviews conducted with the aid of a semi-structured questionnaire, as outlined in (MOEST 2012). The sample size for households was determined using the formula developed by (Arkin & Colton 1963) as:

Sample size, $n = \frac{NZ^2P(1-P)}{Nd^2+Z^2P(1-P)}$

Where,

n=sample size

N=total number of households

Z=confidence level (at 95% level Z=1.96)

p= estimated population proportion (0.5, this maximizes the sample size),

d=error level of 8%

This methodology ensured a representative and statistically sound collection of information from the targeted community.

2.3.2 Data Collection

In order to systematically record the evolving dynamics and adaptive strategies within the community, a Key Informant Survey was undertaken. This involved interviewing individuals who possess in-depth knowledge of the community, its changing circumstances, and act as indicators of the community's vulnerability to CC and disasters. The selected key persons were chosen for their comprehensive understanding of the community's context, providing valuable insights into how the community is adapting to and managing the impacts of climate change and disasters.

2.3.3 Focus Group Discussion

In order to gather specific details about changes in livelihood patterns, socio-economic conditions, climatic hazards, agricultural practices, as well as temperature and precipitation levels, a targeted group discussion was organized. This involved engaging with ward members and representatives of social institutions across five wards within Laljhadi RM.

2.3.4 Community Transect Walk

A community transect walk was conducted, encompassing flood plains, community settlements, and agricultural fields, with the aim of observing and collecting information on the state of natural, social, and physical resources. Additionally, observations were made regarding the impacts of climatic disasters, livelihood practices, and the informal perceptions of the local residents.

2.4 Data Analysis

2.4.1 Mann-Kendall statistical trend test

A significant examination was conducted utilizing the Mann-Kendall test. The Mann-Kendall test, a nonparametric statistical trend analysis tool, is extensively applied in the assessment of trends in climatological (Mavromatis *et al*. 2011) and hydrological time series (Yue & Wang 2004). This test relies on the computation of Kendall's tau, a measure of association between two samples derived from the ranks within the samples. The Mann-Kendall statistical test was executed using Addinsoft's XLSTAT 2014 Software. The null hypothesis was assessed at a 95% confidence level for both temperature and precipitation data, encompassing both annual and seasonal temperature and precipitation data.

2.4.2 Vulnerability Assessment

The primary determinants of vulnerability encompass exposure, sensitivity, and adaptive capacity (NAPA 2010, GoN/MOE). In line with the definition provided by the Intergovernmental Panel on Climate Change (IPCC 2014), this study measures vulnerability as follows:

$$\text{Vulnerability (V)} = \text{f}\,(S * E * \tfrac{1}{A})$$

Where, S = Sensitivity, which is the degree to which a system is affected, either adversely or beneficially, by climate-related stimuli; E= Exposure of system; A= Adaptive capacity of a system

In this study, exposure refers to the nature, extent, and pace of climate variations at the local level. The assessment of CC exposure considered various factors, including temperature, rainfall, hazards, proxy indicators (plants and animals), and physical changes observed over the past 30 years. Exposure was examined at two levels: the community level, through focus group discussions, and the household level, through household surveys. Sensitivity, as explored in this study, relates to the impact of local CC and associated hazards on both local biophysical and socioeconomic systems. The adaptive capacity of a system plays a crucial role in its ability to adapt to CC and mitigate its impacts. A community's effectiveness in adjusting to and moderating the impacts of CC is directly related to its resource endowment, access, and control over resources.

2.4.3 Computation of Index

The evaluation involved the creation of indices for key contributing factors, namely Exposure, Sensitivity, and Adaptive Capacity. Indicators for each factor were assessed on a scale of 1-5, with 1 indicating very low, 2 for low, 3 for medium, 4 for high, and 5 for very high perception based on the IPCC-AR5 report (IPCC 2014). The individual indicator rankings were averaged to derive sub-indices for the components of the major variable. Subsequently, these sub-indices were averaged to obtain the major variable indices (E, S, and A) as per the IPCC-prescribed function. The resulting vulnerability value (V) was categorized into five groups: 0.2-5.04 for very low, 5.04-10.08 for low, 10.08-15.12 for medium, 15.12-20.16 for high, and 20.16-25 for very high vulnerability. Following the determination of vulnerability (comprising Adaptive Capacity, Sensitivity, and Exposure indices), an overall vulnerability index map for the study sites was generated using ArcGIS 10.2.1 software. Additionally, a graph illustrating the results was plotted using OriginPro2023b software.

3. RESULTS AND DISCUSSION

3.1 Social and Economic Status

Out of the 145 respondents, 72.4% were male, with the remaining 27.6% being female. The RanaTharu community emerged as the predominant group, followed by Brahmin/Chhetri and Dalit, constituting 79.5%, 14.6%, and the remainder, respectively. The majority of respondents resided in single-family households, with an average family size of 6.3 members. Regarding literacy, 64.8% of the respondents were literate, with a breakdown of 67.1% for males and 32.9% for females. Conversely, the study revealed that 35.2% of the respondents were classified as illiterate. Agriculture played a pivotal role in the subsistence livelihoods of most respondents, aligning with the national context where over 80% of the population is engaged in agriculture, contributing 33% to the GDP (ADB 2009). In the study area, the primary sources of income for respondents were agriculture (88.17%) and government service (5.6%), while a smaller percentage engaged in business (4.7%) and other activities (1.53%). A few respondents relied on foreign employment in India. Notably, a majority of households demonstrated food sufficiency for an average of eight months per year.

The respondents land holdings were divided into three categories as small (<0.338 hectares), medium (0.338-1.69 hectares), and large (>1.69 hectares). The analysis revealed that the majority of respondents fell into the medium landowner category (0.338-1.69 hectares), with an average landholding size of 0.846 hectares per household. Approximately 76% of respondents engaged in cropland production, and all cultivated lands were located along the banks of the Doda River.

The primary crops cultivated included paddy, wheat, maize, potato, millet, and various cereals. Paddy and maize production emerged as the principal sources of income. Firewood constituted the primary energy source for the community at 81.4%, supplemented by Liquefied Petroleum Gas (LPG) at 13.6% and biogas at 5.0%. Interestingly, 62.9% of the population had not heard about CC. Regarding land cover classification in the study area, agricultural land covered approximately 6655 hectares (43.2%), water bodies accounted for 1153 hectares (7.5%), settlement areas encompassed 1026 hectares (6.7%), and forest land covered 6580 hectares (42.7%).

Observations regarding economic status suggest a significant correlation with adaptive capacity. Individuals or communities with higher economic standing often exhibit greater resilience and adaptability to changes. This is because they often have access to things like education, healthcare, and technology, which help them adapt to different situations. Conversely, those with lower economic status face increased challenges in adapting to changes due to limited resources and opportunities. People from marginalized groups, often associated with specific castes or ethnicities, may face additional barriers in accessing resources and opportunities, limiting their adaptive capacity. Economic status, caste, and ethnicity all play a role in determining how well individuals/communities can adapt to different situations. However, adaptive capacity is not solely determined by caste or ethnicity but influenced by various factors such as access to education, social support networks, and government policies aimed at promoting equality and inclusion.

3.2 Trend Analysis of Climatic Parameters

3.2.1 Temperature Analysis

The analysis of mean temperature over a period of 34 years, from 1985 to 2019, based on seasons revealed an upward trend in temperatures for the pre-monsoon, monsoon, and post-monsoon seasons, while the winter

season exhibited the lowest rate of change (-0.27 °C/decade). Specifically, the pre-monsoon season demonstrated a temperature increase at a rate of 0.2 °C/decade. The annual mean maximum temperature exhibited the highest positive Kendall's tau with the year, measuring 0.045 (Table 2).

According to NCVST (2009), the projections indicate that the pre-monsoon temperature is expected to rise by 1.7 °C by 2030, 3.1 °C by the 2060s, and 5.4 °C by the 2090s. Similarly, the monsoon temperature is projected to increase by 1.4 °C by the 2030s, 2.5 °C by the 2060s, and 4.5 °C by the 2090s. Additionally, the post-monsoon temperature is anticipated to rise by 1.2 °C by the 2030s, 2.6 °C by the 2060s, and 4.6 °C by the 2090s.

Table 2: Summary of the statistical analysis of maximum temperature

Variables	Winter	Pre-Monsoon	Monsoon	Post-Monsoon	Annual
Minimum (°C)	20.2	31.8	32.5	27.7	28.7
Maximum (°C)	25.1	36.6	35.1	31.2	31.6
Mean (°C)	22.8	34.5	33.8	29.6	30.6
Std. deviation	1.097	0.936	0.612	0.650	0.602
Kendall's tau	-0.213	0.126	0.150	0.066	0.045
Kendall's Statistics	-127	75	89	39	27
p-value	0.074	0.293	0.211	0.589	0.712
Trend (°C/decade)	-0.270	0.200	0.090	0.050	0.050
Sen's slope	-0.033	0.014	0.012	0.005	0.003
Sen's Intercept	89.092	6.664	9.511	20.068	24.404
Response	Decrease	Increase	Increase	Increase	Increase
Remarks	Insignificant	Insignificant	Insignificant	Insignificant	Insignificant

Table 3 summarizes the statistical analysis of minimum temperature across different seasons in study area. In winter, the minimum temperature averages at 6.1 °C with a standard deviation of 0.858, showing a slight increasing trend of 0.310 °C per decade. However, this trend is statistically insignificant with a p-value of 0.038. Conversely, during the pre-monsoon and annual periods, there›s a significant increase in minimum temperature, with values of 16.5 °C and 15.8 °C respectively, and corresponding trends of 0.460 °C and 0.280 °C per decade. Both periods exhibit significant positive Kendall›s tau and p-values less than 0.01. Monsoon and post-monsoon seasons, with minimum temperatures of 24.4 °C and 13.5 °C respectively, show insignificant trends and Kendall›s tau values close to zero, indicating no significant changes. However, it›s worth noting that the pre-monsoon and annual periods demonstrate a notable upward trend in minimum temperature, while the others show no significant trend.

In the prior research conducted in the Kanchanpur district, Bhatta (2011) illustrates that the average yearly temperature in the region rise by 0.012 °C per year. Additionally, the mean minimum temperature experienced an increase of 0.026 °C per year, whereas the mean maximum temperature saw a slight decrease of -0.001 °C per year. Furthermore, Kattel and Yao (2013) noted that the warming trend was more pronounced in maximum temperatures, with minimum temperatures displaying greater variability, including positive, negative, or no changes.

Table 3: Summary of the statistical analysis of minimum temperature

Variables	Winter	Pre-Monsoon	Monsoon	Post-Monsoon	Annual
Minimum (°C)	6.1	16.5	24.4	13.5	15.8
Maximum (°C)	9.8	19.5	26.0	18.3	19.1
Mean (°C)	8.4	17.8	25.2	15.8	17.6
Std. deviation	0.858	0.837	0.332	1.050	0.653
Kendall's tau	0.247	0.408	0.005	0.257	0.425
Kendall's Statistics	147	243	3	153	253
p-value	0.038	0.001	0.977	0.031	0.004
Trend (°C/decade)	0.310	0.460	0.010	0.350	0.280
Sen's slope:	0.030	0.049	0.000	0.032	0.038
Sen's Intercept	-52.392	-80.688	24.544	-48.396	-58.018
Response	Increase	Increase	Increase	Increase	Increase
Remarks	Insignificant	Significant	Insignificant	Insignificant	Significant

3.2.2 Precipitation Analysis

The analysis of precipitation over a period of 34 years, from 1985 to 2019, indicated monsoon season contributes the highest proportion of rainfall among all seasons, accounting for about 85.4% of the total precipitation. In contrast, the pre-monsoon and winter seasons contribute 3.46% and 4.94%, respectively, while post-monsoon rainfall is the lowest, accounting for about 6.21% of the total rainfall (Table 4). In Nepal overall, the monsoon season received 79.6% of the total annual precipitation, whereas the pre-monsoon, post-monsoon, and winter seasons received only 12.7%, 4.2%, and 3.5%, respectively (Marahatta et al. 2009).

The annual rainfall data reveals significant fluctuations in the study area, indicating unpredictability in yearly precipitation. According to MoPE (2004), the average trend for Nepal suggests a decreasing annual average precipitation at a rate of 9.8mm per decade. The projected mean annual precipitation for Nepal does not exhibit a clear trend, with variations of -34 to +22% by the 2030s, -36 to +67% by the 2060s, and -43 to +80% by the 2090s (NCVST 2009).

Table 4: Summary of statistical analysis of rainfall from 1985 to 2019

Rainfall	Annual	Winter	Pre-Monsoon	Monsoon	Post-Monsoon
Mean (mm)	3460	143	180	2469	100
Standard Deviation	913	104	116	738	196
Maximum (mm)	5358	352	476	3764	1131
Minimum (mm)	1449	11	17	1085	0
Kendall's tau	0.18	-0.04	0.02	0.14	0.03
Kendall's Statistics	107	-25	11	85	20
p-value	0.13	0.73	0.89	0.23	0.79
Trend (mm/year)	25.47	-0.41	0.72	17.59	1.45
Sen's slope	25.927	-0.632	0.289	18.175	0.115
Sen's Intercept	-48562.682	1378.664	-428.089	-33921.850	-204.723
Response	Increase	Decrease	Increase	Increase	Increase
Remarks	Insignificant	Insignificant	Insignificant	Insignificant	Insignificant

3.3 Adaptation Practices

In the study area, it was observed that local communities employed a variety of autonomous adaptation practices, followed by planned adaptation. Broadly, the adaptation strategies identified through interviews in the study site indicated that households in the six wards experienced water stress induced by climate variability, particularly in relation to floods. More than 80% of the RanaTharu community settlements were located in Laljhadi RM. These adaptation practices were rooted in indigenous knowledge, with some receiving support from various governmental and non-governmental programs. To address changes in the seasonal calendar caused by climate change, farmers had to adjust the timing of crop plantations. The villagers also experimented with hybrid and improved seeds from the local market to enhance production speed and quantity. Hybrid seeds were predominantly used for vegetables and staple crops, especially when home-stored seeds were insufficient. However, many farmers lacked awareness regarding the quality of seeds available in the market. Proper care, recent tools, techniques, and fertilization were essential for the successful growth of hybrid species. Some off-season crops were cultivated throughout the year. Despite the shift to new seeds for increased yield, farmers expressed concerns about the loss of nutritional value and taste in their product.

The autonomous adaptations observed in the study area reflect a distinct form of traditional knowledge and indigenous practices, echoing findings similar to those reported by Maharjan et al. (2011) in their studies conducted in Kailali and Kanchanpur districts.

Villagers and the local government has used the use of biological dams and plantations along the riverside to prevent the cutting of river embankments. In Laljhadi RM, each household has installed taps with the assistance of the local government and the Nepal Red Cross. Another adaptive measure taken by the villagers in response to unpredictable rainfall patterns involves the conservation of forests and the planting of perennial plants around water springs to safeguard water sources.

Due to delayed seasonal rainfall, crops are sowed a month later than the scheduled time. In certain areas of the river basin, the cultivation of water stress-tolerant crops such as groundnut, tomato, sweet potato, garlic, cabbage, bitter gourd, cucumber, and watermelon is becoming increasingly common, meeting the needs of local farmers. The local government, cooperatives, and community-based organizations has conducted training and awareness programs in the villages to promote a blend of traditional and modern farming practices and storage of seeds on the second floor of their houses and in elevated areas within buildings. Additionally, some wards receive seed bins and technical support for local seed production. In response to climate change, some communities have switched from cultivating rice to maize in certain fields, while others have adapted by growing early maturing rice cultivars such as Chaite-4, Chaini, Hardinath, Radha-4, Anjana, and Nimoi to mitigate the impact of floods.

The adaptation practices observed in the study area are closely connected with the social and economic status of the local communities. The predominant reliance on agriculture as the primary source of income, with a majority of respondents engaged in cropland production and cultivating lands along the riverbanks, underscores the close link between livelihood strategies and adaptation efforts. Additionally, the distribution of landholdings into small, medium, and large categories reflects disparities in economic resources, influencing the ability to adopt certain adaptation measures such as experimenting with hybrid seeds or implementing conservation practices. Furthermore, the prevalence of traditional energy sources like firewood among households, coupled with varying literacy rates, highlights the need for tailored awareness and support programs to enhance adaptive capacities across different socioeconomic levels.

3.4 Vulnerability assessment of the community

3.4.1 Adaptive Capacity

The adaptive capacity of each ward was assessed by averaging key indicators, namely the availability of natural resources, demographic factors, physical infrastructure, and the presence of social and financial institutions within the ward. The measured adaptive capacity varied between 2.01 and 3.68, with the highest capacity observed in ward 3 and the lowest in wards 1 and 6. The primary factors influencing community adaptive capacity were identified as knowledge of CC and the availability of infrastructure facilities. While a significant portion of respondents were affiliated with social institutions, only a small number were knowledgeable about CC and had access to communication means. Physical infrastructure, representing the tangible resources, plays a crucial

role in enhancing adaptive capacity. Social capital indicators, encompassing individual networks and mutual trust, are essential for dealing with climate impacts (Ludena & Yoon 2015).

Furthermore, a majority of individuals relied on agriculture and daily wages for their livelihood, with a limited number engaged in government service and business. Notably, ward 3 exhibited a comparatively higher per capita income. At the local level, adaptive capacity is influenced by factors such as access to political power, specific beliefs, and cultural customs *(Cutter et al. 2000)*.

3.4.2 Exposure Index

Climate exposure indicators encompass temperature rise, heavy precipitation, drought, and sea-level rise (IPCC 2014). The exposure index was formulated by averaging these four key indicators, with temperature fluctuations, precipitation patterns, the presence of indicator species, and the incidence of climate-induced disasters serving as primary criteria for its calculation within the community. The resultant exposure index ranged from 2.35 to 3.87. An index based on respondents' perceptions indicated that wards 1 and 6 exhibited the lowest vulnerability to climate change, while ward 3 demonstrated the highest exposure. This heightened exposure in ward 3 can be attributed to the altered course of the Doda River, which now divides the ward into two nearly equal halves. Riverside wards were notably more vulnerable to climate-induced disasters, particularly flood events, exacerbated by the river's change in path.

In the lowland region of Nepal, especially in the Terai flatland where rivers from higher elevations converge, the frequency of flood-related events and fatalities is highest (Pradhan & Shrestha 2007). Within Laljhadi RM, the Doda River experiences routine riverbank erosion and flooding during the rainy season. This has become an integral part of life for the local residents, who face persistent challenges in commuting to nearby villages due to the absence of a bridge over the river. Moreover, the river's altered course contributes to continuous erosion of lands near human settlements, affecting agricultural lands in villages such as Balmi, Dunga, Chandrapur, and Parsiya. The introduction of invasive species, including Ageratum houstonianum and fall armyworm, poses an additional threat to biodiversity in agriculture, river basin areas, and forests. These invasive species are gradually displacing native counterparts, impacting the ecosystem in the long run.

3.4.3 Sensitivity Index

The sensitivity index for each ward in Laljhadi RM was determined by averaging six key indicators. These indicators included agriculture and food security, forest and biodiversity, human settlement, water availability, energy demand, and the gender ratio of the area. The calculation aimed to assess the community's overall sensitivity to various factors. Across the rural municipality, the sensitivity index ranged from 3.15 to 3.75. Ward 4 attained the highest sensitivity score, while Ward 1 registered the lowest value. Notably, the sensitivity was observed to be particularly elevated in wards situated in close proximity to the Doda River.

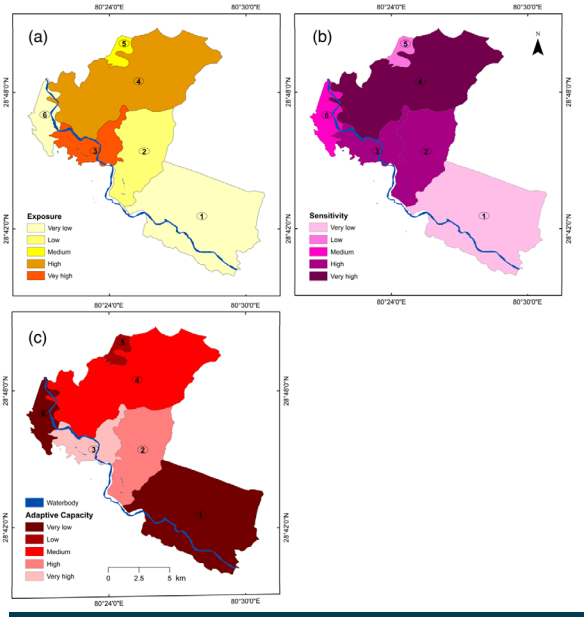


Fig. 2: Map showing the scored vulnerability indices in Laljhadi RM (a) Adaptive Capacity (b) Exposure, and (c) Sensitivity

The heightened sensitivity to climate change in wards 3 and 4 is attributed to human settlements along the riverside and the reduction of forest resources. Our study, which considers vulnerability as a function of exposure, sensitivity, and adaptive capacity, underscores that the primary contributor to vulnerability in Laljhadi RM is the inadequate adaptive capacity, followed by climatic exposure, as detailed in Fig. 3. This finding aligns with a similar study on the spatial assessment of population vulnerability to climate change in Nepal, conducted by (Mainali & Pricope 2017). Their research revealed a

distinct vulnerability pattern, with higher vulnerability observed in the western and northwestern regions of Nepal. Despite the Doda River coursing through ward 1, the residents there exhibit lower sensitivity to floods. This is attributed to the continual displacement experienced by locals since the 2008 flood, prompting many to relocate to forested areas, thereby reducing their vulnerability to flood events. The adverse impacts of climatic risks and hazards, particularly floods, riverbank erosion, and windstorms, are notably severe on infrastructure such as buildings, bridges, roads, foot trails, etc.

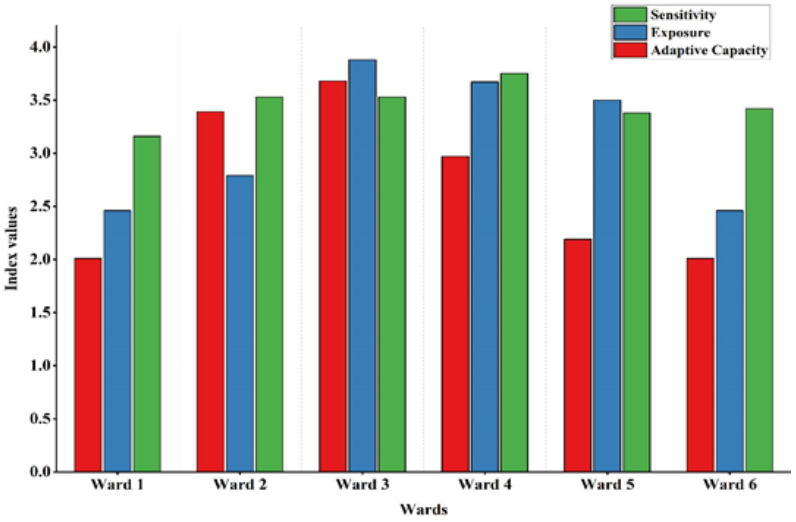


Fig. 3: Result of vulnerability components: adaptive capacity, exposure, and sensitivity of six wards of Laljhadi RM

3.4.4 Vulnerability map

Vulnerability assessments were done to identify and understand the most vulnerable wards of Laljhadi RM based on ranking the components of Adaptive capacity, Exposure, and Sensitivity. The vulnerability index of six wards showed a very high exposure and low adaptive capacity of the area which increased the vulnerability of the area. The highest vulnerability was in ward 3 and it was comparatively least in ward 1 as shown in Table 5.

Table 5: Vulnerability of six wards of Laljhadi RM

Ward	Vulnerability score	Rank
1	5.2	6th
2	11.1	3rd
3	16.8	1st
4	13.6	2nd
5	8.7	4th
6	5.6	5th

The research focused on conducting a vulnerability assessment grounded in community knowledge. By employing participatory methodologies, the aim was to comprehensively address the intricacies of individuals' lives, beginning with their comprehension of the situation, the information available to them, and various factors including local knowledge, personal experiences, skills, household composition in terms of gender and age, and existing coping mechanisms. These factors collectively influence how people adapt to changes in order to mitigate risks and shape their perception of risk, as articulated by Slovic (1992).

The high exposure in lowland Terai is due to the high coefficient of variation of precipitation and a higher rate of temperature increase regionally. The spatial distribution of annual average precipitation aligns with the noted higher coefficient of variation in monsoon rainfall in Nepal, as highlighted by Duncan *et al.* (2013). Given the low-lying nature of this area, recurrent flooding becomes a prevalent occurrence during periods of rainfall. The vulnerability map for Laljhadi RM is depicted in the following Fig. 4.

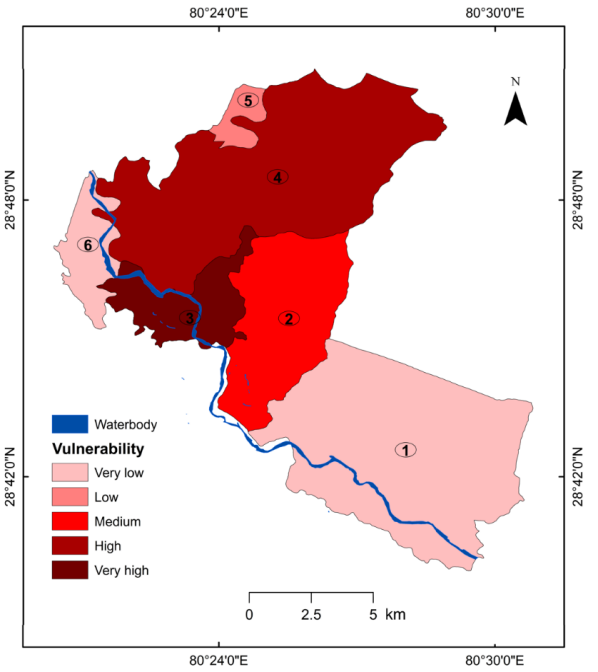


Fig. 4: Map showing the vulnerability of Laljhadi RM

The impact of floods is far-reaching, encompassing the devastation of lives, livelihoods, and critical infrastructure. In a parallel manner, feeder roads and embankments have also been obliterated by the floodwaters. Essential elements of community life, such as the provision of drinking water and electricity, the integrity of schools, and the stability of public buildings, have crumbled in the face of this natural disaster. Furthermore, the flood has resulted in the loss of numerous private residences, properties, domestic animals, and standing crops, all of which have been swept away by the force of the flood. The outcome of these floods is marked by extensive damage, affecting not only individual well-being but also the foundational components that sustain communities.

Key factors that affect how well a community can adapt to climate change include understanding climate change and having the right infrastructure in place. Physical infrastructure, like buildings and roads, is especially important because it provides tangible resources needed to bounce back from challenges. Other factors that influence adaptive capacity are having political influence, cultural beliefs, and traditions. This shows that adapting to climate change involves a variety of different aspects within communities.

Vulnerability assessment reveals that inadequate adaptive capacity, coupled with climatic exposure, contributes significantly to vulnerability within the community. Specifically, factors such as limited knowledge of climate change, infrastructure deficiencies, and economic disparities exacerbate vulnerability. For instance, wards situated along riverbanks face heightened exposure to climate-induced disasters like floods, compounded by inadequate infrastructure and economic resources. Sensitivity to climate change is particularly elevated in areas with human settlements near riversides, emphasizing the interconnectedness of socioeconomic factors and vulnerability.

In the face of significant vulnerabilities, the community has demonstrated resilience through a variety of adaptation practices. These encompass both indigenous knowledge-based strategies and planned adaptations facilitated by governmental and non-governmental initiatives. Farmers have responded to these challenges by diversifying their crops, focusing on alternative cash crops in riverside areas, and adopting early-maturing hybrid varieties to mitigate flood risks. Villagers and local authorities have also taken short-term measures like constructing biological dams and establishing plantations along riverbanks to prevent erosion. However, persistent challenges, such as seed quality concerns and the diminishing nutritional value of crops, underscore the necessity for targeted awareness programs and supportive mechanisms. The interplay of socioeconomic factors, climatic exposure, and adaptive capacity underscores the complex dynamics influencing community vulnerability and resilience. While economic disparities and infrastructure deficiencies pose challenges, community-driven adaptation practices and collaborative efforts offer pathways towards building adaptive capacity and mitigating climate risks.

4. Conclusion

The socio-economic analysis reveals that most respondents belong to the RanaTharu community, primarily engaged in agriculture with notable disparities in literacy rates, landholding sizes, and income sources. Economic status directly affects adaptive capacity, with wealthier individuals or communities exhibiting greater resilience. Conversely, those with lower economic status, especially marginalized groups, face heightened challenges in adaptation due to limited resources. Climatic trends, especially rising temperatures and erratic precipitation, pose significant challenges for

agriculture and water management, exacerbating vulnerability.

Moreover, the study area has experienced significant climate shifts affecting local livelihoods, including reduced rainfall frequency, increased floods, prolonged dry spells, delayed monsoons, and intense late summer rains. Recent years have observed an increase in hot summer days and relatively cold winter days, indicating temperature extremes. This climatic variability has adversely impacted community livelihood resources, particularly in riverside wards facing higher vulnerability. Continuous flooding since 2008 has led to community displacement, forcing people to shift to elevated areas, affecting their sensitivity to flood events. Agricultural land near rivers suffers from inundation and deposition of sand and residue, rendering it unproductive.

Traditional farming practices are threatened to CC, leading farmers to cultivate alternative cash crops in riverside areas and adopt early-maturing hybrid crops to escape floods. To prevent river embankment cutting, villagers and local governments employ biological dams and plantations along the riverside. While short-term adaptation practices have been implemented, the communities lack additional financial resources to address long-term CC challenges. There is a growing interest in modern farming technology, but location-specific adaptation technologies require investment in research at both higher and community levels. Vulnerability assessments show low adaptive capacity across all wards, with specific vulnerabilities varying by hazard and community. Wards 3, 4, and 2 of Laljhadi RM exhibit the highest vulnerability. This research emphasizes that overall exposure to climatic stresses may remain consistent across small-scale settlements, but hazard-specific exposure varies. Communities reliant on natural resources for food, income, and water, such as the Rana and Tharu communities, are highly sensitive to climate-induced hazards. This underscores the importance of community-driven approaches in fostering resilience to climate change.

Acknowledgments

The authors express heartfelt gratitude to the Nepal Academy of Science and Technology (NAST) for providing financial support for the execution of this research study.

References

Arkin, and R. Colton, 1963. *Table of Statistics*. New York: Barnes and Noble.

Aryal, S., G. Cockfield, and T. N. Maraseni, 2014. Vulnerability of Himalayan transhumant communities to climate change. *Climatic Change*, *125*(2), 193–208. DOI: 10.1007/s10584-014-1157-5

Bhatta, R. P., 2011. Climate change impacts on and its adaptation strategies of rural community of Krishnapur VDC in Mohanasubwatershed, Kanchanpur district. *A project paper submitted as a partial fulfillment of the requirement for the degree of Master of Science in watershed management*.

Bista, R. B., 2023. Assessment of Climate Change induced Household Vulnerability in Nepal: Application of Index Method. *ARRUS Journal of Social Sciences and Humanities*, *3*(1), 9–20. DOI: 10.35877/soshum1151

Cutter, S., J. Mitchell, and, M. Scott, 2000. Revealing the Vulnerability of People and Places: A Case Study of Georgetown County, South Carolina. Annals of the Association of American Geographers, 90(4), 713-737. DOI: 10.1111/0004-5608.00219

Cutter, S., J. Mitchell, and, M. Scott, 2000. Revealing the Vulnerability of People and Places: A Case Study of Georgetown County, South Carolina. *Annals of the Association of American Geographers*, 90(4), 713-737. DOI: 10.1016/j.gloenvcha.2008.07.013

Dixit, A., M. Karki, and A. Shukla, 2015. *Vulnerability and Impacts Assessment for Adaptation Planning in Panchase Mountain Ecological Region*, Nepal, Kathmandu, Nepal:

Dixon, J. L., and L. C. Stringer, 2015. Towards a theoretical grounding of climate resilience assessments for smallholder farming systems in Sub-Saharan Africa. Resources 4 (1), 128–154 ISSN 2079-9276 DOI: 10.3390/resources4010128

Duncan, J. M., E. M. Biggs, J. Dash, and P. M. Atkinson, 2013. Spatio-temporal trends in precipitation and their implications for water resources management in climate sensitive Nepal. Applied Geography, 43, 138-146doi: DOI: 10.1016/j.apgeog.2013.06.011

E. K. Pradhan, E. K., and S. K. Shrestha, 2007. Risk of flood-related mortality in Nepal. Disasters, 31(1), 57-70.
DOI: 10.1111/j.1467-7717.2007.00340.
PMID:17367374

Fritzsche, K., S. Schneiderbauer, P. Bubeck, S. Kienberger, M. Buth, M. Zebisch, and W. Kahlenborn, 2014. The vulnerability sourcebook concept and guidelines for standardised vulnerability assessments, Institute for applied Remote Sensing, Department of Geoinformatics, *GIS Austria* Government of Nepal, United Nations Environment Programme, United Nations Development Programme, International Union for Conservation of Nature, German Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety and Institute for Social and Environmental Transition-Nepal

Gentle, P., and T. N. Maraseni, 2012. Climate change, poverty and livelihoods: Adaptation practices by rural mountain communities in Nepal. *Environmental Science and Policy*, *21*, 24–34. DOI: 10.1016/j.envsci.2012.03.007

Giri, M., G. Bista, P. K. Singh, and R. Pandey, 2021. Climate change vulnerability assessment of urban informal settlers in Nepal, a least developed country. Journal of Cleaner Production, 307(April), 127213.
DOI: 10.1016/j.jclepro.2021.127213

IPCC, 2007. Climate Change 2007: *Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*.

IPCC, 2014. *Climate Change: Impacts, adaptation and vulnerability. Summary for policymakers. Contribution of working group II to the fifth assessment report of the intergovernmental panel on climate change*.

Kattel, D. B., T. Yao, K. Yang, L. Tian, G. Yang, and D. Joswiak, 2013. Temperature lapse rate in complex mountain terrain on the southern slope of the central Himalayas. *Theoretical and applied climatology*, *113*(3-4), 671-682.
DOI: 10.1007/s00704-012-0816-6

Kayastha, R. B., W. K. Lee, N. Shrestha, and S. W. Wang, 2023. Assessing the Livelihood Vulnerability of Nomads to Changing Climate in the Third Pole Region of Nepal. *Land*, *12*(5).
DOI: 10.3390/land12051105

Ludena, C. E., and S. Yoon, 2015. *Local Vulnerability Indicators and Adaptation to Climate Change; A Survey*. Washington DC: Inter-American Development Bank, Technical Note No. 857.
DOI: 10.18235/0009259

Maharjan, S. K., E. R. Sigdel, B. R. Sthapit, and B. R. Regmi, 2011. Tharu community's perception on climate changes and their adaptive initiations to withstand its impacts in Western Terai of Nepal. *Academic Journal*, 6(2), 35-42.

Mainali, J., and N. G. Pricope, 2017. High-resolution spatial assessment of population vulnerability to climate change in Nepal. *Applied Geography*, 82, 66-82.
DOI: 10.1016/j.apgeog.2017.03.008

Mavromatis, T., and D. Stathis, 2011. Response of the water balance in Greece to temperature and precipitation trends. *Theoretical and Applied Climatology*, *104*(1-2), 13-24.
DOI: 10.1007/s00704-010-0320-9

McCarthy, J. J., O. F. Canziani, N. A. Leary, D. J. Dokken, and K. S. White, 2001. *Climate Change 2001: Impacts, Adaptation, and Vulnerability Contribution of Working Group II to the Third Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, Cambridge, UK.

MOE, 2010. Climate Change Vulnerability mapping for Nepal, *Ministry of Environment, GON*, Kathmandu, Nepal.

MoEST, 2012. *Field Guidelines for CBVA Tools and Methodologies Users*, Ministry of Environmental Science and Technology.

NAPA, 2010. *National adaptation programme of action (NAPA) to climate change*. Kathmandu: Ministry of Environment, Government of Nepal.

NCVST, 2009. *Vulnerability through the eyes of vulnerable: Climate change induced uncertainties and Nepal's development predicaments. Kathmandu and Boulder, Colorado: Institute for Social and Environmental Transition-Nepal (ISET-Nepal) and Institute for Social and Environmental*

Transition (ISET) for Nepal Climate Vulnerability Study Team.

Oxfam, 2009. *Even the Himalayas Have Stopped Smiling. Climate Change, Poverty, and Adaptation in Nepal*. Kathmandu, Nepal: Oxfam International.

Pandey, R., and S. K. Jha, 2012. Climate vulnerability index - measure of climate change vulnerability to communities: A case of rural Lower Himalaya, India. *Mitigation and Adaptation Strategies for Global Change*, *17*(5), 487–506. DOI: 10.1007/s11027-011-9338-2

Panthi, J., S. Aryal, P. Dahal, P. Bhandari, N. Y. Krakauer, and V. P. Pandey, 2016. Livelihood vulnerability approach to assessing climate change impacts on mixed agro-livestock smallholders around the Gandaki River Basin in Nepal. *Regional Environmental Change*, *16*(4), 1121–1132. DOI: 10.1007/s10113-015-0833-y

Parry, M., C. Rosenzweig, and M. Livermore, 2005. "Climate change, global food supply and risk of hunger. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 360(1463), 2125-2138. DOI:10.1098/rstb.2005.1751 PMID:16433098 PMCID:PMC1569580

Poudel, S., S. Funakawa, H. Shinjo, and B. Mishra, 2020. Understanding households' livelihood vulnerability to climate change in the Lamjung district of Nepal. *Environment, Development and Sustainability*, *22*(8), 8159–8182. DOI: 10.1007/s10668-019-00566-3

Reed, M. S., and L. C. Stringer, 2016. Land degradation, desertification and climate change: Anticipating, assessing and adapting to future change. Routledge, UK PB: 978-1- 84971-271-2. DOI: 10.4324/9780203071151

Slovic, P., 1992. *Perception of risk: Reflection on the psychometric paradigm*. New York: Social Theories of Risk.

Sujakhu, N. M., S. Ranjitkar, J. He, D. Schmidt-Vogt, Y. Su, and J. Xu, 2019. Assessing the livelihood vulnerability of rural indigenous households to climate changes in Central Nepal, Himalaya. *Sustainability (Switzerland)*, *11*(10). DOI: 10.3390/su11102977

Thakuri, S., S. Dahal, D. Shrestha, N. Guyennon, E. Romano, N. Colombo, and F. Salerno, 2019. Elevation-dependent warming of maximum air temperature in Nepal during 1976-2015. *Atmospheric Research*, 261-269. DOI: 10.1016/j.atmosres.2019.06.006

Yohe, G., and R. Tole, 2002. Indicators for social and economic coping capacity-moving toward a working definition of adaptive capacity. *Global Environmental Change*, 12(1), 25-40. DOI: 10.1016/S0959-3780(01)00026-7

Yue, S., and C. Wang, 2004. The Mann-Kendall test modified by effective sample size to detect trend in serially correlated hydrological series. *Water resources management*, *18*(3), 201-218. DOI:10.1023/B:WARM.0000043140.61082.60

Isolation of Peroxidase Enzyme from Various Vegetables and Coupling to IgG

P. Hemalatha[1], K. Shivani[1], M. Godavari[1], T. Sri Vidhya[1], S. Manju Devi[1] and A. Sai Padma[1*]

[1]Department of Biochemistry & Nutrition, Bhavan's Vivekananda College of Science, Humanities and Commerce, Sainikpuri, Secunderabad, Telangana, India

***CORRESPONDING AUTHOR:**

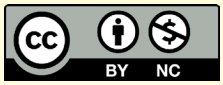
A. Sai Padma
Email:saipadmabhavans@yahoo.co.in

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI :
https://doi.org/10.3126/njst.v21i2.67171

Date of Submission: Feb 8, 2023

Date of Acceptance: Apr 20, 2024



ABSTRACT

Coupling of IgG with peroxidase enzyme finds various applications in determining the antigen-antibody interactions, in diagnostics or for research purpose. In the present study, Peroxidase enzyme was isolated from various vegetables like tomato (*Solanum lycopersicum),* cabbage *(Brassica oleracea)* and Radish *(Raphanus sativus) a*nd partial purification of enzymes was done with ammonium sulphate fractionation to 80% saturation. The active peroxidase enzyme was coupled to Goat anti-human IgG and the efficiency of coupling procedure was tested against Protein A sepharose purified human IgG. The coupling procedure was done by using sodium periodate and coupling was confirmed by dot blot and ELISA procedures. The present study provides an indigenous method to isolate an active peroxidase enzyme and couple to various IgG and use in immuno detection methods at very cost effective manner.

Key words: Antibody coupling, Dot Blot, ELISA, Human IgG, Periodate, Peroxidase

1. Introduction

Peroxidases (EC.1.11.1.7) are heme-containing enzymes belonging to oxidoreductases. They are hydrogen peroxide decomposing enzymes associated with oxidation of the broad range of phenolic and non-phenolic substrates. Plant peroxidases have an essential physiological role in the growth and development of plant throughout its life cycle. Due to the versatility of peroxidases during reaction and their ubiquitous nature, they have potential applications in various immunological, medicinal, biotechnological, and industrial sectors (Pandey *et al.* 2017; Pandey & Dwivedi 2015; de Oliveira 2021). Plant peroxidases from various sources such as Ficus, lettuce, citrus, broccoli, royal palm, soybean, papaya, wheat grass, Solanum melongena, lemon etc. have been isolated, purified and characterized (Pandey *et al.* 2017. Chandrasekaran *et al.* 2014). Peroxidase enzyme isolated from various sources have been used in immunoassays, diagnostic test kits, wastewater treatment and soil remediation (Chukwudi *et al.* 2021). Immobilization of peroxidase enzyme has gained much attention in the construction of biosensors due to economical and viability in enzyme immunoassays (Shivakumar *et al.* 2017). The proficiency

of peroxidases to yield chromogenic products at low concentrations makes them compatible enzymes for the preparation of enzyme-linked immunosorbent assay (ELISA) kits, which are used in the diagnosis of several diseases (Singh 2019). Due to the peroxidase ability to yield chromogenic products at low concentrations and its relatively good stability, it is well-suited for the preparation of enzyme conjugated antibodies and application in diagnostic kits and as well peroxidases are widely used in clinical biochemistry and enzyme immunoassays (Krell 1991; Vamos-Vigyazo 1981; Lin 1996). Among all peroxidases isolated, horse radish peroxidases are most generally used for the analytical purposes (Idesa & Getachew 2018). However, other plant peroxidases having extensive pH and temperature stability are emerging as choice for HRP (Tijseen 1985).

Human Immunoglobulin G (IgG) can be purified from different human serum samples by Protein A sepharose column chromatography (Klaus Huse 2002). Conjugation of IgG – Peroxidase enzyme involves formation of stable, covalent linkages between the enzyme and antigen-specific monoclonal or polyclonal antibody (IgG). This involves various steps to produce the IgG-Peroxidase conjugate. The coupling reaction which produces a conjugate that resembles the native protein include, Sodium periodate oxidation method, Maleimide-sulfhydryl method, Cyanuric chloride methods (Weinryb 1968; Nakane & Kawaoi 1974; Jackson *et al.* 1987; Hosoda *et al.* 1985; Presentini & Terrana 1995).

2. Materials & Methods

Protein-A sepharose affinity column (2 mL) kit, Human IgG, Goat anti-human IgG, Goat anti-human IgG- HRP were purchased from Bangalore Genei, India. All other chemicals were from Qualigens, India.

2.1. Isolation and characterization of peroxidase from vegetable sources

2.1.1. Sample collection and preparation of enzyme extract

Vegetables (Tomato, Cabbage, Radish) used for this study were intentionally selected and purchased from local market. After the preliminary procedures like washing, peeling and slicing, 50 g of each vegetable slices were crushed with pestle and mortar to obtain homogenous sample. 200 mL of distilled water was added separately to all the crushed substances and homogenized in a mechanical blender for 15 minutes and centrifuged at 6000 rpm for 15 minutes in a high speed cooling centrifuge. The supernatant was filtered through cheese cloth and the sediment was discarded. To selectively inactivate the contaminating traces of the catalase moieties, the supernatant was heated to 65 °C for 3 minutes in a water bath and cooled immediately by placing on ice bath for 30 minutes. After thermal inactivation, the samples containing crude enzyme were preserved at -20 °C until further use.

2.2. Partial purification of peroxidase enzyme by ammonium sulphate fractionation

The crude enzyme preparation was subjected to ammonium sulphate fractionation to 80% saturation under constant ice cold conditions. The precipitated protein was collected by centrifugation at 12000 rpm for 30 minutes. The precipitate was dissolved in small volume of 100 mM potassium phosphate buffer, pH 6 and dialyzed against same buffer for overnight under low temperature. The dialyzed fractions were analysed for protein content by Lowry's method (Lowry *et al.* 1955).

2.3. Peroxidase enzyme assay

To a clean, glass calorimeter tube, the following solutions were added one after other and as follows-1.7 mL of glass distilled water, 0.32 mL of 0.1 M potassium phosphate buffer (pH 6.0), 0.16 ml of 0.50% (v/v) hydrogen peroxide solution and 0.32 mL of 5% (w/v) pyrogallol solution. The reaction mixture was mixed by inversion and equilibrated at 20 °C in an incubator for 10 minutes. Suitable aliquots (0.4 mL) of enzyme solution was added to the reaction mixture and the solution was mixed thoroughly by inversion and the increase in A_{420} was recorded for 3 minutes. The $\Delta A_{420/30\ secs}$ were obtained using the maximum linear rate for all the tests and blanks (Shannon *et al*. 1966).

The Pyrogallol which was used as the substrate in the assay when combined with hydrogen peroxide gets converted to purpurogallin in the presence of peroxidase. The chromogenic reaction development of the enzyme activity was determined calorimetrically.

Unit – One unit of peroxidase will form 1 mg of purpurogallin from pyrogallol in 30 seconds at pH 6 at 20 °C.

$$\text{Units/ml enzyme} = \frac{(A_{420/30sec}\ \text{Test sample} - A_{420/30sec}\ \text{Blank})\ (3)\ (df)}{(12)\ (0.4)}$$

Where

- df = Dilution factor (4)
- 3 = Volume (in mL) of assay
- 12 = Extinction coefficient of 1mg/ml of purpurogallin at 420 nm.
- 0.4 = Volume (in mL) of enzyme used.

2.4. Effect of temperature on peroxidase:

To study the effect of temperature on peroxidase enzyme, the assay conditions were maintained under the study as described earlier, except that the temperatures were altered. The various temperatures used in the study were 20 °C, 40 °C, 60 °C and 80 °C.

2.5. Effect of pH on peroxidase

To study the effect of pH on peroxidase, the assay conditions were maintained under the study as described earlier, except that the pH of the buffer was altered. The phosphate buffer of 100 mM with various pH (pH-3,4,5,6,7,8) was used for the assay and temperature was maintained at 20 °C for all the tubes.

2.6. Effect of substrate on peroxidase

To study the effect of substrate on peroxidase, the assay conditions were maintained under the study as described earlier, except that the substrate concentration was altered. The various pyrogallol substrate concentrations used were (0.1%,0.2%,0.3%,0.4%,0.5%) at 20 °C and 100 mM potassium phosphate buffer, pH 6.0.

2.7. Preparation of Human serum

Human blood samples were procured from students of age group (21-22 years) with prior consent after explaining them for the reason to be used in the research project.

After collecting the whole blood, the blood was allowed to get clotted by leaving undisturbed at room temperature for 30 min-1 hour. Clots were removed by centrifuging at 3000 rpm for 10 minutes in a clinical centrifuge. The resulting serum (supernatant) was transferred into clean Eppendorf tubes and stored at –20 °C until further use.

2.8. Purification of IgG from Human serum by Protein A-Sepharose Column

Protein-A affinity Sepharose column Kit was procured from GeNei, Bangalore, India. The column was equilibrated with 10 bed volumes of 1X equilibration buffer and it was allowed to drain through the column. Serum was diluted with (1:10) in 1X Equilibration Buffer. Diluted Serum samples were loaded to the equilibrated Protein A Column. The column was washed with 25 bed volumes of 1X Equilibration Buffer and eluted with 5 bed volumes of 1X elution buffer as 1 ml fractions into eppendorf tubes, each tube containing 25 µl of neutralizing buffer. The absorbance of the eluted fractions was measured at 280 nm in a UV-VIS spectrophotometer. Graph was plotted with fraction number versus absorbance values. Fractions with highest absorbance were pooled and protein content was determined by Lowry's method (Lowry *et al.* 1955).

2.9. Determination of Purity of IgG by SDS-PAGE

Purity of IgG was checked on SDS-PAGE (Lamelli 1970) by mixing the fractions having highest absorbance with 2 X sample buffer and boiled for 5 minutes on a boiling water bath. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R250 (Brunelle Julie and Green Rachel 2014) and by Silver staining method Chevallet *et al*. (2006).

2.10. Molecular weight determination

The R_f values were calculated for the standard proteins and for heavy and light chains of IgG and molecular weight was determined by plotting the graph with log (MW) as a function of R_f. The equation $y = mx + b$ was used to determine the MW of the unknown proteins.

2.11. Confirmation of IgG by Western Blot & Dot blot

The purified IgG from Protein A Sepharose affinity column was confirmed by western blotting following the method developed by Towbin *et al.* (1979). After blotting onto the nitrocellulose membrane by electro transfer method, the membrane was blocked with 5% casein and finally incubated with commercial Goat anti-human IgG – HRP (1:100 dilution) and bands were developed by staining TMB/H_2O_2 substrate

For dot blot procedure, 20 µl of purified human IgG (1 mg/mL) was spotted on nitrocellulose membrane strips along with controls. The membranes were air dried and incubated with goat anti-human IgG – HRP (1:500 dilution) solution for 1 hour. Substrate (TMB/H_2O_2) was added to all the membranes and the spots were developed (Faoro *et al.* 2011; Oprandy *et al.* 1988).

2.12. Coupling of IgG with Peroxidase enzyme

Conjugation of Goat anti-human IgG with purified peroxidase was performed by periodate method developed by Pavliuchenko *et al.* with some modifications (Pavliuchenko *et al.* 2019). Freshly prepared 300 µl of 0.1 M Sodium periodate was added to 1500 µl of Peroxidase enzyme (1 mg/mL). The mixture was stirred gently on shaker for 20 minutes at room temperature in dark. 500 µl of 1 mM Sodium acetate buffer, pH 4.4 was added to the above mixture and pH was adjusted to 9.5 by adding 0.2 M Sodium carbonate buffer, pH 9.5. Goat anti-human IgG solution (250 µl) was added to the activated peroxidase solution. Mixture was stirred gently on shaker for 2 hrs at room temperature. 150 µl of freshly prepared 0.1 M Sodium borohydride solution was added to peroxidase - IgG mixture. The solution was incubated with Sodium borohydride for 90 mins at room temperature with gentle shaking. The sample was dialyzed against 1 X PBS (pH 7.2) under cold conditions for 7-8 hours. The goat anti-human IgG –peroxidase conjugate collected after dialysis was subjected to confirmatory tests by dot blot and ELISA tests.

2.13. Confirmation of IgG-peroxidase conjugation: In order to determine the efficiency of coupling, the following procedures are were used

2.13.1. Dot blot

Purified human IgG samples of 20 µl were spotted on nitrocellulose membrane strips. After air drying the membranes for 5 minutes, the membranes were incubated with freshly coupled goat anti-human IgG-peroxidase for 1 hour. The blots were developed with TMB/H_2O_2 and observed for the coloured spots. In the control spot, the IgG was incubated with commercial goat anti-human IgG-HRP and the blank spot was incubated with distilled water.

2.13.2. ELISA

Sandwitch ELISA kit from GeNei, Bangalore was used for all the buffers to use in the current procedure. Test wells were loaded with 200 µl of purified human IgG which was mixed with coating buffer in 1:2 ratio along with control wells having the same antigen. The plate was incubated for 1 hr at 37 °C in a temperature regulated incubator. 200 µl of blocking buffer was added to all the wells and further incubated at room temperature for 1 hr. The wells were rinsed for 3 times with distilled water and the water was discarded completely. 200 µl of freshly coupled goat anti-human IgG-peroxidase conjugate was added to all test wells and 200 µl of commercial goat anti-human IgG-HRP conjugate was added to all control wells. The plates were incubated at room temperature for 1 hr. Unbound antibodies were discarded by plate inversion and wells were rinsed for 3 times with 1X wash buffer. 200 µl of Substrate TMB/H_2O_2 was added to all wells, test and control and incubated for 10 mins at room temperature for colour development. Stop solution of 100 µl was added to all the wells. The contents of each well were transferred completely to individual test tubes containing 2 mL of 1X stop solution. The substrate blank was prepared by adding 200 µl of 1X TMB/H_2O_2 to 2.1 mL of 1X stop solution. Absorbance was read at 450 nm in a UV-VIS spectrophotometer (Voller *et al.* 1978). The experiment was repeated twice to confirm the results.

3. Results

3.1. Protein content and enzyme activity

The peroxidase enzyme isolated from various vegetable sources of Tomato, Cabbage and Radish have shown good protein yield after ammonium sulphate fractionation and exhibited high enzyme activity with substrate pyrogallol. The protein fraction containing peroxidase enzyme isolated from Tomato is having protein concentration of 10 mg/mL as determined by Lowry's method and enzyme activity of 2.275 U/ml. The peroxidise isolated from Cabbage has shown 20 mg/mL protein and 2.675 U/mL enzyme activity, whereas from Raddish it was found as 8 mg/mL protein and 2.125 U/mL enzyme activity respectively. The intensity of the yellow-orange colour obtained during the assay reaction further matches with the concentration of enzyme in the preparations.

3.2. Effect of temperature on enzyme activity

The optimum temperature for enzyme extracted from sources cabbage and tomato is at 60 °C whereas radish has showed burst of activity at 22 ^{0}C and again at 60 ^{0}C showed varied nature of enzyme (Fig. 1)

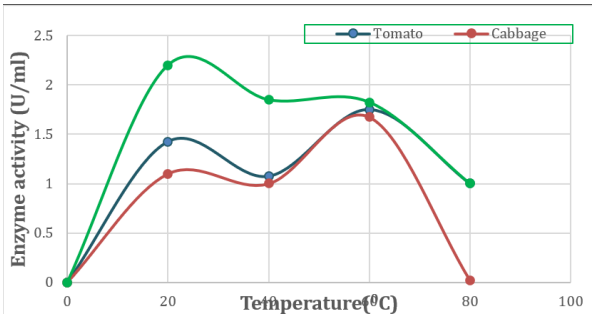


Fig. 1: Effect of temperature on Peroxidase activity

3.3. Effect of pH on enzyme activity

All the enzymes from sources tomato, cabbage and radish have shown broad range activity from pH 4-8. Radish has shown consistently high activity compared to other two (Fig. 2).

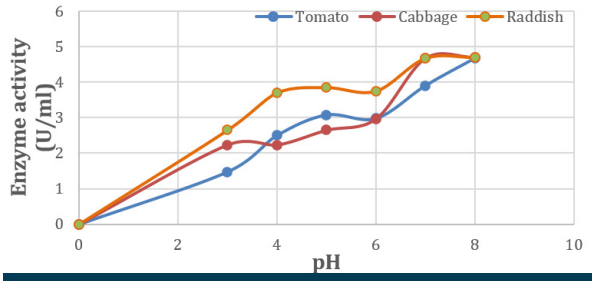


Fig. 2: Effect of pH on Peroxidase activity

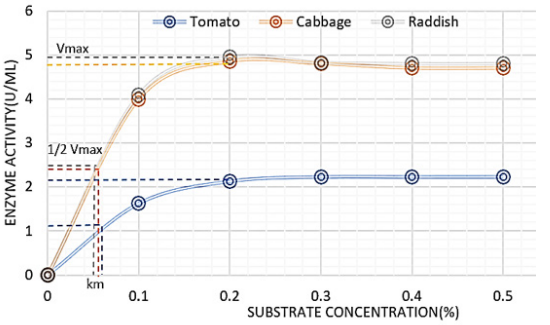


Fig. 3: Effect of substrate concentration on peroxidase activity

3.4. Effect of substrate concentration on enzyme activity

To study the effect of substrate on peroxidase, the assay conditions were maintained under the study as described earlier, except that the substrate concentration was altered. The various pyrogallol substrate concentrations used were (0.1%,0.2%,0.3%,0.4%,0.5%) at 20 °C and 100 mM potassium phosphate buffer, pH 6.0. V_{max} and K_M values were determined for all the enzymes and the values were found to be for Tomato 2.225 U/ml and 0.7 mg/ml; for Cabbage 4.85 U/mL and 0.6 mg/mL; for Radish 4.95 U/mL and 0.55 mg/mL respectively of V_{max} and K_M .

3.5. Purification of IgG from human serum by Protein A Column Chromatography

IgG from three different human serum samples were purified on protein-A sepharose column. The peak fraction number was different amongst all three samples as indicated in Fig. 4. The reason would be due to different affinities of IgG with protein A amongst different samples.

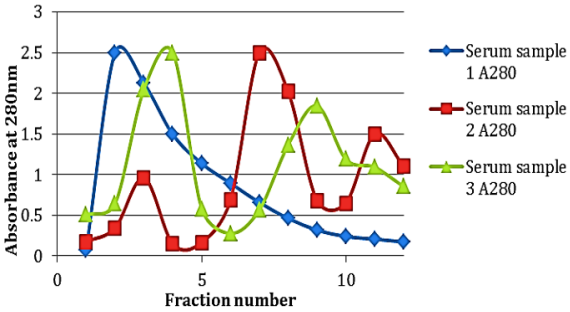


Fig. 4: Elution profile of IgG from serum samples on Protein A-Sepharaose column

3.6.SDS-PAGE

The purity of the IgG was checked on 12% gels under denaturing conditions by SDS-PAGE method and bands were visualized by both Coomassie Brilliant Blue R250 staining and silver staining procedures (Fig.s. 5 and 6). The heavy and light chains were appeared as pure bands. Little high molecular weight bands were visualized in silver stained gels, indicating aggregates of IgG.

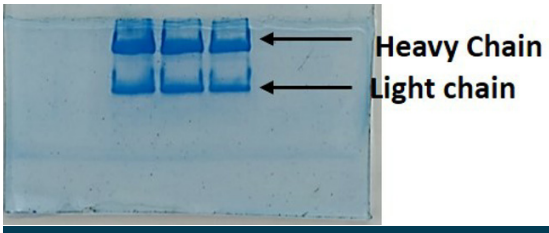


Fig. 5: Analysis of IgG by SDS-PAGE

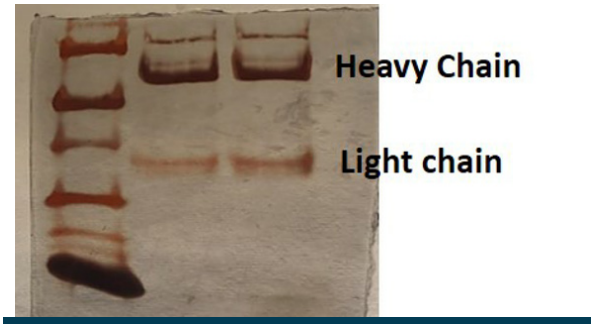


Fig. 6: Analysis of IgG by silver staining

3.7. Molecular weight determination

The molecular weight of heavy and light chains was determined and found to be 50 kDa and 23.5 kDa respectively (Fig. 7) and matching with the standard reports. R_f = migration distance of the protein

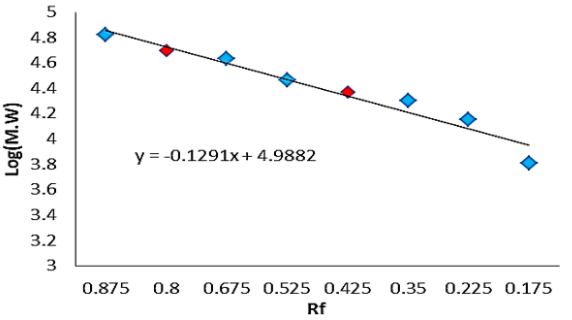


Fig. 7: Molecular Weight Determination by SDS-PAGE

3.8. Western blot Analysis of IgG

The purified human IgG was confirmed by western blot technique and found it was cross-reactive with commercial Goat anti- human IgG-HRP. Both heavy chain and light chain reacted with the antibodies and stained with substrate TMB/H_2O_2 (Fig. 8).

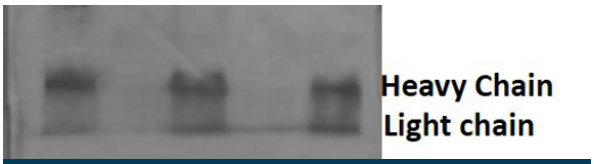


Fig. 8: Analysis of IgG by Western Blot

3.9. Dot blot

The isolated IgG was further subjected to dot blot analysis by using commercial Goat anti- human IgG- HRP antibodies and blue dots observed on the nitrocellulose membrane, further confirming the isolated protein was human IgG. Control antigen used was commercial human IgG from GeNei. Bangalore (Fig. 9).

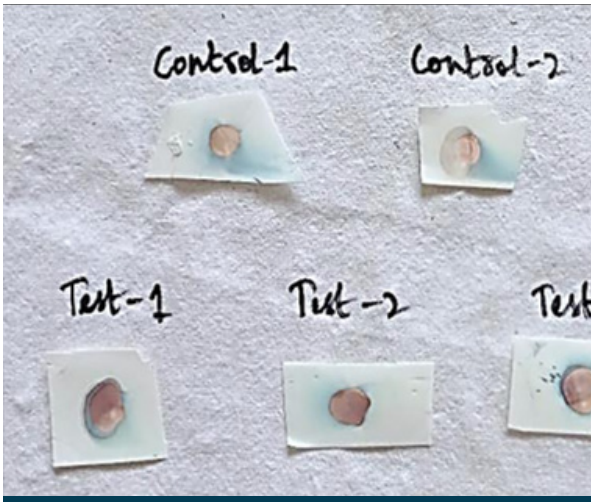


Fig. 9: Dot blot analysis of IgG

3.10. Confirmation of coupling of peroxidase to Goat anti -human IgG

3.10.1. Dot blot

The coupling efficiency of goat anti-human IgG-peroxidase was tested against purified human IgG by dot blot method and results clearly indicated the coupling procedure was efficient by the appearance of blue spots on nitrocellulose membrane (Fig. 10).

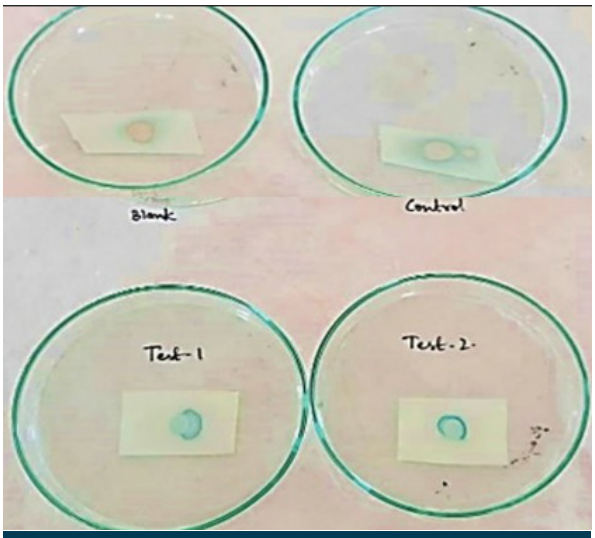


Fig. 10: Analysis of coupling reaction by Dot blot

3.10.2. ELISA

Another confirmatory test for the coupling procedure was by ELISA method. The ELISA plate wells were coated with antigen purified human IgG and incubated with coupled Goat anti- human IgG- peroxidase conjugate. Control also was run with commercial antibodies of Goat anti human IgG- HRP. The coloured wells of the test (B well) indicated comparatively high color intensity and clearly confirms efficient coupling between Goat anti-human IgG and peroxidase enzyme from vegetable sources (Fig. 11).

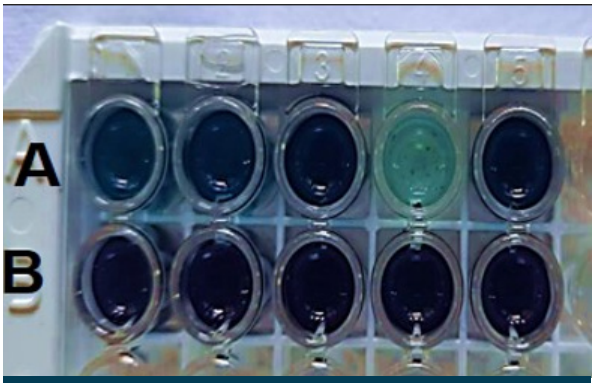


Fig. 11: Analysis of coupling reaction by ELISA

4. Discussion

Peroxidase enzyme has great ability to produce stable chromogenic product and is a suitable enzyme in various diagnostic kits based on enzyme-conjugated antibody technology (Pandey *et al*. 2017; Shivakumar *et al.* 2017). The present study is directed to prepare Peroxidase –IgG conjugate with high thermal stability, strong binding affinity for the substrate compared with high-cost commercial HRP-conjugate. Extraction and partial purification of peroxidase enzyme from tomato, cabbage and radish was done very cost-effectively with simple laboratory procedures. Thermal stability of the enzymes was observed as very high as the enzymes have shown good activity starting from temperature 40 ^{0}C and continued till 80 ^{0}C. This indicates that the enzymes are suitable for storage for long time usage. These results are similar to the reported ones of litchi peroxidase and asparagus (Mizobutsi *et al.* 2010; Rodrigo *et al.* 1996) suggesting that extensive heat treatments are needed to inactivate the peroxidases and suitable for coupling procedures.

Though several methods were reported for the purification of IgG from serum, a simple, one-step procedure by affinity chromatographic method has shown as a highly suitable and efficient method (Mariam *et al.* 2015). Also, large yield of affinity-purified IgG was reported from protein A Sepharose column (Blanc *et al*. 2009). During coupling process, the periodate solution provided a slightly acidic medium that prevents the self-coupling of peroxidase (Beyzavi *et al.* 1987). Conjugates of radish peroxidase with commercial goat anti-human polyclonal antibodies were produced by a modified process of the previously described periodate oxidation method [26]. Functionality of prepared conjugates was also tested by dot blot and ELISA methods and the results were satisfactory.

5. Conclusions

The present study explained the coupling procedure of Peroxidase enzyme, isolated from Tomato (*Solanum lycopersicum),* Cabbage *(Brassica oleracea)* and Radish *(Raphanus sativus)* with Goat anti-human IgG. The coupling method involved chemical reagents of sodium periodate and Sodium borohydride. The efficiency of coupling procedure was tested by dot blot and ELISA procedures using human IgG, purified by using Protein A-Sepharose affinity column. The present study provides an indigenous method to isolate an active peroxidase enzyme from various natural sources

and couple to IgG and use in immuno detection methods at very cost effective manner.

Competing Interests

None

Acknowledgments

Authors acknowledge the management and principal of Bhavan's Vivekananda College for providing all necessary infrastructural facilities.

References

Beyzavi, K., S. Hampton, P. Kwasowski, S. Fickling, V. Marks, and R. Clift, 1987. Comparison of horseradish peroxidase and alkaline phosphatase-labelled antibodies in enzyme immunoassays. Annals of Clinical Biochemistry 24, (1987), 145-152.
DOI: 10.1177/000456328702400204
PMID:3035992

Blanc, M. R., A. Anouassi, M. A. Abed, S. Canépa, V. Labas, and G. Bruneau, 2009. A new method to discriminate immunogen-specific heavy-chain homodimer from heterotetramer immunoglobulin G directly in immunized dromedary whole plasma proteins: Western ligand blotting. Veterinary Immunology and Immunopathology Journal127: 340-349.
DOI: 10.1016/j.vetimm.2008.10.334
PMID:19091427

Brunelle Julie, L., and R. Green, 2014. Coomassie Blue Staining. Pages 161-167. In: Lorsch, Jon.. Methods in Enzymology. Academic Press.
DOI: 10.1016/B978-0-12-420119-4.00013-6
PMID:24674070

Chandrasekaran, G., S. K. Choi, Y. C. Lee, G. J. Kim, and H. J. Shin, 2014. Oxidative biodegradation of single-walled carbon nanotubes by partially purified lignin-peroxidase from Sparassis latifolia mushroom. Journal of Industrial and Engineering Chemistry 20:3367-3374.
DOI: 10.1016/j.jiec.2013.12.022

Chevallet, M., S. Luche, and T. Rabilloud, 2006. Silver staining of proteins in polyacrylamide gels. Nature Protocols1: 1852-1858.
DOI: 10.1038/nprot.2006.288
PMID:17487168 PMCID:PMC1971133

Chukwudi, I. N., C. A. Onyetugo, and I. N., Amarachi, 2021. Peroxidase, an example of enzymes with numerous applications. Asian Journal of Chemical Sciences 10:11-22.
DOI: 10.9734/ajocs/2021/v10i219087

de Oliveira, F. K., L. O. Santos, and J. G. Buffon, 2021. Mechanism of action, sources, and application of peroxidases. Food Research International143, 110266,
DOI: 10.1016/j.foodres.2021.110266
PMID:33992367

Faoro, V., and G. Stanta, 2011. Dot Blot in Stanta, G. Editor. Guidelines for Molecular Analysis in Archive Tissues.Pages 275-276. Springer, Berlin, Heidelberg.
DOI: 10.1007/978-3-642-17890-0_44

Hosoda, H., W. Takasaki, S. Aihara, and T. Nambara, 1985. Enzyme labelling of steroids by the N-succinimidyl ester method, Preparation of alkaline phosphatase-labelled antigen for use in enzyme immunoassay. Chemical and pharmaceutical bulletin, 33:5393-5398.
DOI: 10.1248/cpb.33.5393
PMID:3914377

Idesa, G. D., and B. Getachew, 2019. Extraction and Partial Purification of Peroxidase Enzyme from Plant Sources for Antibody Labeling. The Pharma innovation Journal 8: 418-422.

Jackson, J. C., J. L. Charlton, K. Kuzminski, G. M. Lang, and A. H. Sehon, 1987. Synthesis, isolation, and characterization of conjugates of ovalbumin with monomethoxypolyethylene glycol using cyanuric chloride as the coupling agent. Analytical biochemistry, 165:114-127.
DOI: 10.1016/0003-2697(87)90208-9
PMID:3688426

Klaus Huse, Hans-Joachim Böhme, and Gerhard H Scholz, 2002. Purification of antibodies by affinity chromatography. Journal of Biochemical and Biophysical. Methods, 51: 217-231.
DOI: 10.1016/S0165-022X(02)00017-9
PMID:12088882

Krell, H. W., 1991. Peroxidase: An important enzyme for diagnostic test kits. Pages 469-478 in Lobarzewsky, J., Greppin, H., Penel, C., Gaspar, T. Editors. Biochemical, molecular and physiological aspects of plant peroxidases.University M. Curie, Lublind Poland, and University of Geneva, Geneva Switzerland

Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature., 227:680-685.
DOI: 10.1038/227680a0
PMID:5432063

Lin, Z. F., L. H. Chen, and W. Q. Zhang, 1996. Peroxidase from I. Cairica (L) SW. Isolation, purification and some properties. Process Biochemistry 5: 443-448.
DOI: 10.1016/0032-9592(95)00088-7

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193: 265-275.
DOI: 10.1016/S0021-9258(19)52451-6
PMID:14907713

Mariam, S. H. S., C. W. Ooi, W. S. Tan, O. A. Janna, A. Arbakariya, and B. T Tey, 2015. Purification of rabbit polyclonal immunoglobulin G with ammonium sulphate precipitation and mixed-mode chromatography. Separation and Purification Technology Journal 144: 133 - 138.
DOI: 10.1016/j.seppur.2015.02.012

Mizobutsi, G. P., F. L. Finger, R. A. Ribeiro, R. Puschmann, L. Ludmila, and F. M. Wagner, 2010. Effect of pH and temperature on peroxidase polyphenol oxidase activities of litchi pericarp, *Sci. Agric* 67: 213.
DOI: 10.1590/S0103-90162010000200013

Nakane, P. K., and A. Kawaoi, 1974. Peroxidase-labeled antibody, A new method of conjugation. Journal of histochemistry and cytochemistry, 22:1084-1091.
DOI: 10.1177/22.12.1084
PMID:4443551

Oprandy, J. J., J. G. Olson, and T. W. Scott, 1988. A rapid dot immunoassay for the detection of serum antibodies to eastern equine encephalomyelitis and St. Louis encephalitis viruses in sentinel chickens. American Journal of Tropical Medicine and Hygiene, 38:181.
DOI: 10.4269/ajtmh.1988.38.181
PMID:2829636

Pandey, V. P., and U. N. Dwivedi, 2015. A ripening-associated peroxidase from papaya having a role in defense and lignification: Heterologous expression and *in-silico* and *in-vitro* experimental validation. Gene555:438–447.
DOI: 10.1016/j.gene.2014.11.013
PMID:25447898

Pavliuchenko, N., V. Hazarnian, and M. Bassil, 2019. Modification of Periodate Oxidation Method to Produce HRP-IgG Conjugate and Test its Stability Overtime. American Journal of Molecular Biology, 9: 52-63.
DOI: 10.4236/ajmb.2019.92005

Pandey, V. P., M. Awasthi, S. Singh, S. Tiwari, and U. N. Dwivedi, 2017. A comprehensive review on function and application of plant peroxidases. Biochemistry and Analytical. Biochemistry. 6: 1-16.
DOI: 10.4172/2161-1009.1000308

Presentini, R., and B. Terrana, 1995. Influence of the antibody peroxidase coupling methods on the conjugates stability and on the methodologies for the preservation of the activity in time. Journal of immunoassay 16:309-324.
DOI: 10.1080/15321819508013565
PMID:7593652

Singh, R. S., T. Singh, and A. Pandey, 2019. Microbial Enzymes- An Overview. Pages 1-40 in Singh, Ram Sarup Singhania, Reeta Rani, Ashok Pandey, Larroche Christian. Advances in Enzyme Technology. Elsevier, Amsterdam, New York.
DOI: 10.1016/B978-0-444-64114-4.00001-7

Rodrigo, C., M. Rodrigo, A. Alvarruiz, and A. Frígola, 1996. Thermal inactivation at high temperatures and regeneration of green asparagus peroxidase. Journal of Food Protection 59:1065-1071.
DOI: 10.4315/0362-028X-59.10.1065
PMID:31195467

Shannon, L., E. Kay, and J. 1966. Lew, Peroxidase isozymes from horseradish roots, Isolation and physical properties. Journal of Biological Chemistry 241:2166,
DOI: 10.1016/S0021-9258(18)96680-9
PMID:5946638

Shivakumar, A., B. G. Jashmitha, and M. R. Dhruvaraj, 2017. Role of Peroxidase in Clinical Assays: A Short Review. Journal of Clinical Nutrition and Dietetics 3: 1921.
DOI: 10.4172/2472-1921.100048

Tijseen, P., 1985. Properties and preparation of enzymes used in enzyme immunoassays. Pages 173-219in Laboratory techniques in biochemistry and molecular biology, practice and theory of enzyme immunoassays. Elsevier, Amsterdam, New York.
DOI: 10.1016/S0075-7535(08)70140-2

Towbin, H., T. Staehelin, and J. Gordon, 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications, Proceedings of Natl]ional Academy of Sciences *U S A.*76:4350.
DOI: 10.1073/pnas.76.9.4350
PMID:388439 PMCID:PMC411572

Vamos-Vigyazo, L., 1981. Polyphenol oxidase and peroxidase in fruits and vegetables, CRC Critical Reviews in Food Science and Nutrition. 15:49-127.
DOI: 10.1080/10408398109527312
PMID:6794984

Voller, A., A. Bartlett, and D. E. Bidwell, 1978. Enzyme immunoassays with special reference to ELISA techniques, *J Clin Pathol*., 31: 507-520.
DOI: 10.1136/jcp.31.6.507
PMID:78929 PMCID:PMC1145337

Weinryb, I., 1968. The oxidation of horseradish peroxidase by periodate. Biochemical and .Biophysical Research communications., 31:110-112.
DOI: 10.1016/0006-291X(68)90038-7
PMID:4296876

Study of the Properties of Coconut Fiber to explore Suitability to be used in Reinforced Concrete

Khem Raj Joshi[1*], Bharat Mandal[2] and Tarumay Ghoshal[3]

[1]Department of Civil Engineering, DIT University, Uttarakhand, India
[2]Department of Civil Engineering, TU Institute of Engineering, Pulchowk Campus, Nepal
[3]Department of Civil Engineering, DIT University, Uttarakhand, India

***CORRESPONDING AUTHOR:**
Khem Raj Joshi
Email:khemjoshi1978@gmail.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI :
https://doi.org/10.3126/njst.v21i2.64634

Date of Submission: Aug 4, 2024

Date of Acceptance: Oct 13,2024

ABSTRACT

Natural fibers have been utilized in construction materials consistent with the concept of sustainability. These fiber sources are abundant in many parts of the world. It is established that the orientation of coconut fiber used in concrete mix, attempts to obtain the highest possible ductility as opposed to normal brittle nature of concrete. The use of these fibers increases tension capacity of that concrete and reduces negative environmental consequences. Therefore, in this study, physical as well as mechanical properties of coconut coir have been evaluated to explore the use of it as favorable concrete reinforcing fiber. It is found that coconut fibers have 0.2 to 0.4 times tensile strength of mild steel. Similarly, its elongation property against mild steel is near about 0.3 times before breaking. The other major property, bonding with concrete is also found comparable to bond of reinforcement with concrete. The non-toxic and environmental friendly properties are particularly additional advantages on the use of such bio composites in concrete.

Keywords: Coconut fiber, mortar, fiber treatment, mechanical characteristics, and durability.

1. Introduction

The current high economic growth has greatly increased the demand for building materials such as concrete, so most countries allocate a significant portion of their budgets to building infrastructure. Concrete, a major building material, is very important for the development of infrastructure such as roads, bridges and buildings. The consumption of concrete worldwide is estimated to be around 23 billion tons per year. This makes cement and concrete one of the most commonly used materials in construction (Huda & Alam 2014; Meyer 2009; Tam *et al.* 2018; Kylili & Fokaides 2017). In fact, it is estimated that the production of concrete accounts for around 8% of the world's carbon dioxide emissions. Therefore, increased use of unconventional, natural, recycled and alternative building and insulation materials supports the sustainability of the built environment (Kylili & Fokaides 2017). Green or alternative

materials are increasingly being investigated for their benefits. Using natural fibers in reinforced composites reduces the environmental impact and reliance on traditional materials to make concrete. Moreover, the amount of natural fibers has increased significantly in recent decades. Farmers collect millions of tons of natural fiber each year from a variety of crops such as cotton, abaca, sisal, coconut, jute, hemp, flax and ramie, and livestock such as sheep, goats, camels and alpacas. The use of natural fibers is beneficial for at least five good reasons, including environment protection, cost savings, social responsibility, sustainable development and high-tech performance.

Conventional concrete (CC) often exhibits compressive strength but weak tensile strength. Steel rods are commonly used to correct this failure in conventional concrete, also known as reinforced cement concrete. Fiber reinforced concrete (FRC) is a special type of concrete created when fibers are added to concrete to increase its natural tensile strength (Cosgun 2016; Fediuk 2018; Sekar & Kandasamy 2019; Yin *et al.* 2019). Due to their sustainability, biodegradability, and environmental considerations, natural fibers are now the most commonly used, along with secondary cement components. It is one of the reinforcing materials, but natural fibers also help to reduce CO_2 emissions. Natural fibers are cheaper, stiffer and easier to recycle than synthetic fibers and also available anywhere in the world.

It is well known that properties of concrete can be changed by adding different organic and inorganic material depending on the situation to make it suitable for usage as building material. These properties of conventional concrete: workability, color and time for curing can be altered by changing the constituents. However, researchers often search for traditional materials such as agricultural waste to make concrete environmentally friendly (Alengaram *et al.* 2008; Mannan & Ganapathy 2004; Mannan & Ganapathy 2002; Olanipekun *et al.* 2006). While recycling of such type of building materials, environmental quality will not be degraded further. In this context, coconut fibers can be a wise option.

Coconut cultivation is a popular hobby all over the world, especially in tropical and subtropical regions. According to a recent study (Ohler 1999), over 50 billion coconuts are harvested for coir fiber worldwide. Ripe coconut shells are used to produce coir, a common natural substance used in the manufacturing of durable goods. Coir proving grounds are abundant with coir, which can be used as concrete reinforcement. In addition, it brings income to coconut farmers who benefit from increased demand from the construction industry.

Hence, coconut fibers due to being economic has been an option to be used in concrete and studies have been done to test such concrete from durability and strength aspects. In this context, studies were conducted on durability of slag mortar that was reinforced with coconut fiber (John *et al.* 2005). Fibers were extracted from aged walls and tested. It was found durability was not affected. In similar study for testing fiber reinforced concrete, mechanical properties of such concrete were tested under dry and wet conditions and found less susceptible to sulphate reactions (Sivaraja *et al.* 2010). Another study confirmed that flexural strength in coconut fiber reinforced concrete was enhanced by 90% (Ng *et al.* 2017). Not only this, tensile strength and modulus of elasticity of coconut fiber reinforced concrete is increased compared to concrete without fiber. Also, such use of organic material will not cause any leaching of harmful substances. Studies also reveal that use of coir fibers in concrete prevents micro cracks, especially coconut coir fiber reduces overall weight of concrete due to being low density in nature (Salain *et al.* 2016). However, those studies further suggested that coir fiber length, higher mixing water content are important parameters to consider while using these fibers in concrete (Bharat *et al.* 2018; Pederneiras *et al.* 2021; Yashwanth *et al.* 2021).

Therefore, the purpose of this study is to identify the properties of coir as a fiber reinforcement that can be used in concrete. Both physical and mechanical properties are targeted here for coconut coir fiber. Also, the aim is to explore the suitability of these type of fibers which may then be used in various constructions that do not always necessarily need steel fibers such as canal lining, secondary minor structures etc.

2. Methodology

Coconut coir is a natural fiber extracted from the husk of a coconut. The unprocessed fiber is manually taken from a coconut after the coir has been removed. The method of expelling coconut coir includes few steps. The mature coconuts are gathered from the trees and cleared out to dry under the sun for some days. The outer hard shell of the coconut is removed employing a cleaver or other sharp device. The brown sinewy layer between the hard shell and the coconut meat

is evacuated employing a machine or physically employing a scrubber. The coir fibers are washed altogether to expel any dirt or impurities. The fibers are soaked in water for a number of days to relax those and to make more flexible. The fibers are beaten with a wooden hammer or other device to be partitioned and broken down into little pieces. The strands are spread out to dry within the sun or employing a drying machine until they are totally dry. The dried strands are screened to expel any remaining pollutants or bigger pieces. The coir strands are compressed into bunches for transportation and capacity. Once the coir strands have been prepared, they are prepared to be utilized as a fiber and other reason.

In another way of removing fiber, coconut shell is kept in a tidy space and retted. During the retting process, the coconut shells are buried in moist soil to promote microbial degradation of the softer sections. The shells are then pummeled and cleaned to help readily detach the coir fibers and extract the fibers from the coco peat powder using a sieve. Figure 1 and 2 indicate the fiber extraction process.

Fig.1: Raw Coconut Coir with cell

Fig. 2: Extracted raw coconut coir

From the raw coir around 74% of fiber can be extracted. That indicates that on average, 26% of dust and 74% of fiber remains in coconut coir after the inner hard cell was removed.

In this work, the physical and mechanical characteristics of unprocessed coconut fiber were examined using the accepted measuring practices, which were primarily based on Indian Standards. Following tests were performed: (i) Measurement of Diameter and Length of Fiber, (ii) Bulk Density, (iii) Density, (iii) Water absorption, (iv) Tensile Strength, (v) Bond Strength to concrete.

3. Results and Discussions

(i) Measurement of Diameter and Length of fiber:

Fibre aspect ratio is the ratio of length to diameter of a fibre and is usually expresses as a single number greater than 1. It is an important fiber parameter. Therefore, the fiber diameter was evaluated using a digital thickness gauge and a screw gauge having list count of 0.01mm, and the length was measured with a Vernier caliper. Coir fibers vary in diameter from head to tail. The average fiber diameter of the coir is found 0.4 mm to 0.50 mm. Same dimensions for this type of fibers were used and recommended for coir fibres reinforced concrete (Ali 2011). The average diameter of 20 random samples tested was found 0.45 mm. For calculation length should be at least two times of developing length.

(ii) Bulk Density:

Bulk density is determined by measuring the volume of a known mass of sample in its natural arrangement. The coir is a fibrous material that can be different form therefore bulk density does not mean any value. Therefore, density of the fiber is evaluated in its compressed form. As per the magnitude of compression, the bulk density varies. In average compression by hand, its bulk density was found 189 kg/m^3. Fig. 3 denotes the bulk density measurement.

Calculation:

Diameter of Vessel=6.4 cm, Height of Vessel =25.5 cm, Mass of Fibre =154.5 gm,

Volume of vessel = $(\pi d^2 h)/4$ =819.917 cm^3

Density =Mass /Volume = 0.189 gm/ cm^3

Fig. 3: Bulk Density Measurement by using cylindrical vessel

Fig. 4: Density Measurement using water replacement method

(iii) Density

A standard water displacement method is used to find out density of the fiber. The least count of the balance used for weighing was 1 gm. The density of fiber was found 0.84 gm/cc, that is 16% lighter than water. Being the denser material comparison to other similar fiber, its durability in wet soil is much longer than other organic materials. Fig. 4 is showing density measurement. The results are consistent with the findings in Chauhan & Arya, 2018.

Calculation:

Weight of empty pycnometer = (W1) =227.10 gm., Weight of pycnometer + dry sample = (W2) =252.6 gm., Weight of fiber =W5= W2-W1=25.5 gm, Volume of empty pycnometer = (V1) =1000 cm^3, Volumn of water filled pycnometer= (V2) = 1000 cm^3,Volumn of water replace by fibre from pycnometer =(V3)= 30 cm^3, Volume of fibre = Volume of replaced water = (V4)= 30 cm^3, Density (D)= W5 / V4 = 0.84 gm / cm^3

(iii) Water absorption

One of the most crucial tests on a fiber is its moisture content, which has an impact on the material's physical properties drying at 100 °C. Therefore, coir fiber was put in oven at a temperature of 50 °C for 24 hrs to evaporate any moisture contained in the fiber. The oven dried fiber were put in water for 24 hrs and weighted. The water observation of coir fiber was found 15% in average. Therefore, in average 15% of more water is required if we use fiber in concrete. This test has been recommended by Mittal & Chaudhary 2018; Pandey *et al.* 2016 for evaluation of effects while using coir fiber in reinforced concrete.

Calculation

Moisture Content = (Wet Weight - Dry Weight) / Dry Weight × 100%.

Weight of dry weight of sample W1= 85 gm, Weight of 24 hr. wet weight of sample W2=97.85, Moisture content = (W2-W1)/W1*100 % = 15.12 %

(iv) Tensile Strength

It is very challenging to test a thin fiber's tensile strength. It cannot be clamped in a metal jaw or wedge shaped jaw. As a result, the breaking strength of the fiber is measured by clamping both ends by high strength yarn. Tensile strength is assessed at room temperature under typical conditions. Figure 5 and 6 represent tensile strength measurement.

Fig. 5: Tensile strength testing of fiber

Fig. 6: Measurement of length

A dry fiber's tensile strength was discovered to range from 53.06 N/mm^2 to 108.79 N/mm^2.The samples that were tested had an average strength of 78.39 N/mm^2 (Table 1) The values were found in consistence with the similar study made by Nagarajaiah *et al.* 2024; Widnyana *et al.* 2020. On other words, the breaking loads of fiber were found ranging from 7.70 N to 18.87 N. A fiber in average breaks at a force of 12.8 N. A coconut fiber's tensile strength is roughly one third that of the mild steel's yield strength. Evaluation of fiber elongation took place during the tensile strength test. Elongation was found to be 8% on average. The fact that there is very little variation in the elongation of the samples tested. Using the average strength and elongation as a basis, the average elasticity modulus would be 979.88 N/mm^2. The spring balance of least count 5 gm was used to measure breaking load.

The tensile strength (σ) was calculated by using Equation 1. Where F is force to failure (N), A is a cross sectional area fracture plane normal to fiber axis (m^2).

$$\sigma = F/A \quad \text{..... Eq.1}$$

Table 1: Tensile strength observation values

Sample Length (mm)	Diameter (mm)	Area (mm2)	Force (kg)	Force (N)	Stress (N/mm2)	Average Stress (N/mm2)
50	0.45	0.1590	1.068	10.477	65.91	78.39
50	0.5	0.1963	1.658	16.265	82.88	
50	0.47	0.1734	1.923	18.865	108.79	
50	0.40	0.1256	1.092	10.713	85.29	
50	0.43	0.1451	0.785	7.701	53.06	
50	0.45	0.159	1.500	14.715	92.57	
50	0.46	0.166	1.507	14.784	89.00	
50	0.47	0.173	1.489	14.607	84.24	
50	0.52	0.212	0.987	9.682	45.62	
50	0.49	0.188	1.505	14.764	78.33	
50	0.41	0.132	1.062	10.418	78.95	
50	0.48	0.181	1.402	13.754	76.04	

(v) Bond Strength to concrete.

Length of a fiber imbedded in concrete, depends upon its tensile strength and bonding to concrete. The bonding strength of fibers was evaluated by casting a M20 concrete cube with insertion of different length of fibers in it. After 28 days curing of concrete, the embedded fibers were pulled in the same manner as in testing of tensile strength. Here 10 mm, 15 mm, 20 mm and 25 mm length was embedded in concrete. All the samples were failed by broken fiber without sleeve out of the concrete. Therefore, development length of the coconut fiber should be less than 10 mm for M20 concrete. Taking the 15 mm as development length for M20 concrete, we did show that the bond strength of concrete is nearly equal to that of ribbed reinforcement. Figure 7 and 8 depict bond strength test. The importance of fiber length in reinforced concrete is mentioned in Ahmad *et al.* 2020; Biswas *et al.* 2011.

Fig. 7: Sample for bond test

Fig. 8: Bond strength testing

4. Conclusion

After the evaluation of all tests, it can be concluded that the tensile strength of coconut fiber is in average 78.39 MPa with bond strength similar to ribbed reinforcing bar. It can be used as fiber reinforcement in concrete to enhance tensile property of concrete which is also supported by Widnyana *et al.* 2020, Salain *et al.* 2016. The length of 20 mm would be sufficient as fiber reinforcement. Diameter of a coconut fiber varies from 0.4 mm to 0.5 mm (Ali 2011). These fibers can be used as reinforcing for secondary structures where strength and durability aspects are not primarily focused also to make such structures economic and environmental friendly.

5. References

Alengaram, U. J., M. Z. Jumaat, and H. Mahmud, 2008. Ductility behaviour of reinforced palm kernel shell concrete beams. European Journal of Scientific Research 23(3):406-420. DOI: 10.1201/9780203859926.ch41

Ali, M., 2011. Coconut fibre: A versatile material and its applications, Journal of Civil Engineering and Construction Technology Vol. 2(9), pp. 189-197, 2 September, 2011. Available online at http://www.academicjournals.org/jcect ISSN 2141-2634

Ahmad, W., S. H. Farooq, M. Usman, M. Khan, A. Ahmad, F. Aslam, and M. Sufian, 2020. Effect of coconut fiber length and content on properties of high strength concrete. Materials, 13(5), 1075. DOI: 10.3390/ma13051075

Biswas, S., S. Kindo, and A. Patnaik, 2011. Effect of fiber length on mechanical behavior of coir fiber reinforced epoxy composites. Fibers and Polymers, 12, 73-78. DOI: 10.1007/s12221-011-0073-9

Bharat, M., T. Sushant, G. Subid, and T. Aasish, 2018. Mechanical Properties of Concrete with partial replacement of Coarse aggregates by Coconut Shells and reinforced with Coconut Fibre. Journal of Building Materials and Structures 5(2):227-238. DOI: 10.34118/jbms.v5i2.61

Chauhan, N., and N. Arya, 2018. Coconut fiber: A natural versatile material. International Journal of Chemical Studies, 6(6), 555-561.

Cosgun, T., 2016. An experimental study of RC beams with varying concrete strength classes externally strengthened with CFRP composites. Journal of Engineered Fibers and Fabrics
DOI: 10.1177/155892501601100302

Fediuk, R., 2018. Reducing permeability of fiber concrete using composite binders. Special Topics and Reviews in Porous Media: An International Journal 9(1).
DOI: 10.1615/SpecialTopicsRevPorousMedia.v9.i1.100

Huda, S. B., and M. S. Alam, 2014. Mechanical behavior of three generations of 100% repeated recycled coarse aggregate concrete. Construction and building materials 65:574-582.
DOI: 10.1016/j.conbuildmat.2014.05.010

John, V. M., M. A. Cincotto, C. Sjöström, V. Agopyan, and C. T. D. A. Oliveira, 2005. Durability of slag mortar reinforced with coconut fibre. Cement and Concrete Composites 27(5):565-574.
DOI: 10.1016/j.cemconcomp.2004.09.007

Kylili, A., and P. A. Fokaides, 2017. Policy trends for the sustainability assessment of construction materials: A review. Sustainable Cities and Society 35:280-288.
DOI: 10.1016/j.scs.2017.08.013

Pederneiras, M. C., R. Veiga, and J. de Brito, 2021. Physical and mechanical performance of coir fiber-reinforced rendering mortars. Materials 14(4):823.
DOI: 10.3390/ma14040823

Mannan, M. A., and C. Ganapathy, 2002. Engineering properties of concrete with oil palm shell as coarse aggregate. Construction and Building Materials 16(1):29-34.
DOI: 10.1016/S0950-0618(01)00030-7

Mannan, M. A., and C. Ganapathy, 2004. Concrete from an agricultural waste-oil palm shell (OPS). Building and environment 39(4):441-448.
DOI: 10.1016/j.buildenv.2003.10.007

Meyer, C., 2009. The greening of the concrete industry. Cement and concrete composites 31(8):601-605.
DOI: 10.1016/j.cemconcomp.2008.12.010

Mittal, M., and R. Chaudhary, 2018. Experimental study on the water absorption and surface characteristics of alkali treated pineapple leaf fibre and coconut husk fibre. Int. J. Appl. Eng. Res, 13(15), 12237-12243.

Nagarajaiah, M., A. Yadav, S. Prasannakumar, R. R. Mahadevaiah, and P. Hiremath, 2024. Finite Element Study on Coconut Inflorescence Stem Fiber Composite Panels Subjected to Static Loading. Engineering Proceedings, 59(1), 215.
DOI: 10.3390/engproc2023059215

Ng, G. P., G. S. Urgessa, Y. Bayleyegn, and H. A. R. I. A. N. T. O. Hardjasaputra, 2017. Mechanical Properties of Coconut Fiber-Reinforced Concete. Resilient Structures and Sustainable Construction, Valencia, Spain.
DOI: 10.14455/ISEC.res.2017.93

Ohler, J. H., 1999. Modern Coconut Management; Palm Cultivation and Products. Available online: https://practicalactionpublishing.com/book/1431/modern-coconut-management (March 03, 2024).
DOI: 10.3362/9781780445502

Olanipekun, E. A., K. O. Olusola, and O. Ata, 2006. A comparative study of concrete properties using coconut shell and palm kernel shell as coarse aggregates. Building and environment 41(3): 297-301.
DOI: 10.1016/j.buildenv.2005.01.029

Pandey, M. R., S. Pokharel, and N. K. Karn, 2016. Water absorption study of the coconut coir fibre after surface modification by different chemicals for varied duration. In Proceedings of International Conference on Soil and Environment. Bangalore, India (pp. 22-23).

Salain, I. M. A. K., I. N. Sutarja, N. M. A. Wiryasa, and I. M. Jaya, 2016. Mechanical Properties of Coconut Fiber-Reinforced Concrete. In The 6th International Conference of Asian Concrete Federation pp. 21-24.

Sekar, A., and G. Kandasamy, 2019. Study on durability properties of coconut shell concrete with coconut fiber. Buildings 9(5):107.
DOI: 10.3390/buildings9050107

Sivaraja, M., Kandasamy, N. Velmani, and M. S. Pillai, 2010. Study on durability of natural fibre concrete composites using mechanical strength and microstructural properties. Bulletin of Materials Science 33:719-729.
DOI: 10.1007/s12034-011-0149-6

Tam, V. W., M. Soomro, and A. C. J. Evangelista, 2018. A review of recycled aggregate in concrete applications (2000-2017). Construction and Building materials 172:272-292.
DOI: 10.1016/j.conbuildmat.2018.03.240

Widnyana, A., I. G. Rian, I. W. Surata, and T. G. T. Nindhia, 2020. Tensile Properties of coconut Coir single fiber with alkali treatment and reinforcement effect on unsaturated polyester polymer. Materials Today: Proceedings, 22, 300-305.
DOI: 10.1016/j.matpr.2019.08.155

Yashwanth, M. K., 2021. Evaluation of Compressive Strength of Coir Fibre Reinforced Concrete. Turkish Journal of Computer and Mathematics Education (TURCOMAT) 12(10):68-73.

Yin, S., Y. Yu, and M. Na, 2019. Flexural properties of load-holding reinforced concrete beams strengthened with textile-reinforced concrete under a chloride dry-wet cycle. Journal of Engineered Fibers and Fabrics 14.
DOI: 10.1177/1558925019845902

Guidelines for Manuscript Submission

Submission Preparation Checklist

As part of the submission process, authors are required to check their 'submission's compliance with all of the following items:

- The manuscript neither is previously published nor is it under consideration for another journal
- The text must adhere to the stylistic and bibliographic requirements outlined in the **Guidelines for Author(s)**
- Ensure that the **Declaration/Conflict of Interest and Letter to the Editor** have been submitted along with the manuscript
- A list of 3-5 recommended or opposed reviewers (name, email, affiliation) has been provided in **Letter to Editor**
- Prepare title page as per the **Title Page** guidelines
- High-resolution vector pictures and figures must be submitted separately and placing their low-resolution copies with captions in the manuscript
- Author(s) should provide the responsibilities performed by each author as per the **Author(s)' Contributions**
- List the highlights of your manuscript (not more than five in bullets)
- Submit your final manuscript as per the **Manuscript Structure** with all grammatical and plagiarism checked. Unedited manuscripts are rejected from further consideration.

Guidelines for Author(s)

Author(s) are required to prepare their manuscript stringently following the instructions for authors given below. Failure to follow instructions, manuscripts may be returned to authors without a scientific assessment. Online submission and review of the manuscript are mandatory for all **Types of Manuscript**. During online submission, the author must declare compliance with the **Submission Preparation Checklist**. The submitted manuscript will be subjected to pre-review by reviewers and editors. The manuscript deemed inappropriate will be rejected without a review. Full instruction for online submission can be found here. If you have any queries, please contact the editorial office at njst@nast.org.np.

Type of Manuscript

NJST publishes only the manuscripts related to the following type:

Original Research: They are the manuscripts that present the results of original research by the Author(s). The manuscript should not be more than 6000 words. The word count includes all parts of the manuscript except figures, tables, and captions. The manuscript should be organized as per the **Manuscript Structure:** The abstract of the manuscript should not exceed 250 words. A list of not more than five Keywords (if phrases used then not more than three), excluding words used in the title should be arranged in alphabetical order and separated by a comma and placed beneath the abstract.

Reviews: Reviews are detailed and up-to-date syntheses of topical themes. They often describe previous works and offer new insights to guide future research works. Review articles are usually up to 8000 words. There is no specific format for review articles, butsections should be broken up in sub-headings at Author(s)'s discretion. However, the manuscript organized as per the **Manuscript Structure** for original research is preferable.The abstract of the manuscript should not exceed 200 words. A list of not more than five Keywords (if phrases used then not more than three), excluding words used in the title should be arranged in alphabetical order and separated by a comma and placed beneath the abstract.

Short communication: Short communication provides a forum to disseminate crucial scientific findings that are not enough for the research/original works. Short communication should be a brief contribution up to 3000 words. The manuscript should also be organized as per the structure for original research except for the conclusion. The abstract of the manuscript should not exceed 100 words. A list of not more than five Keywords (if phrases used then not more than three), excluding words used in the title should be arranged in alphabetical order and separated by a comma and placed beneath the abstract.

Perspectives: It provides a forum to present a viewpoint on an emerging issue of science and technology. They should be of broad interest to general people.

Perspectives should be less than 2000 words. There is no specific format for perspective articles, but sections should be broken up in sub-headings at 'author's discretion. The manuscript should be organized as follows: Title, Author(s), their affiliation, and contact address of corresponding author(s), Abstract, Keywords or phrases, Introduction, Materials and Methods, Results and Discussion, Conclusion, Acknowledgment, and References. The abstract of the manuscript should not exceed 200 words. A list of not more than five Keywords (if phrases used then not more than three), excluding words used in the title should be arranged in alphabetical order and separated by a comma and placed beneath the abstract.

Language

Both American and British English are accepted but not of a combination of these. Authors for whom English is a second language should have their manuscript corrected carefully before the submission.

Manuscript Structure

The manuscript should be prepared meticulously in the journal style (e.g., citation, language, etc.). The manuscript should be arranged as follows: Title, Author(s), their affiliation, and contact address of corresponding author(s), Abstract, Keywords or phrases, Introduction, Materials and Methods, Results, Discussion, and Conclusion, Acknowledgment, and References. Tables and low-resolution figures should be embedded in the text and highresolution vector pictures and figures provided in a separate page of the manuscript. The entire manuscript must be doubled-spaced with 12-point font, Times New Roman. Leave a 1 inch (2.54-cm) margin on all sides of each page. All pages of the manuscript should be numbered.

Title Page

1. A concise title
2. A list of author names, affiliation(s), and email addresses (do not provide position) in the order, First name, Middle name, and Last name. If more than one author, separate with a comma then " and" "before the last author
3. Affiliation of the authors numbering 123 after the name in superscript form
4. Astrix (*) after the superscript number to distinguish the corresponding author
5. Name of the affiliated institutions as per the number
6. The name, complete mailing address (including email address, mobile) of the corresponding author
7. A word count of the entire paper, excluding tables and figure legends
8. The number of tables and figures
9. Abstract
10. Keywords
11. Submitted date

Section number

The authors are advised to number the sections up to five levels. The section should be clearly defined and logically ordered. Subsections should be numbered as 1.1, 1.2… similarly, 1.1.1, 1.1.2. Section numbers can be used for cross-referencing in the manuscript. The heading and sub-heading of the manuscript vary according to the Type of Manuscript (e.g., original articles, review, etc.). The tentative structure of the manuscript is given below:

Introduction

Provide a detailed background of your research.

Materials and Methods

A sufficient detailed description of methods should be provided to allow the work to be reproduced. When work is followed from the previously published work, it must be referred to as proper/original citation.

Results

The results should be clear and concise.

Discussion

The discussion should explore the significance of the results of the work without repeating them. A combined Results and Discussion section is often appropriate.

Conclusions

State main conclusions of the study. The conclusion may be standalone or form a subsection of a Discussion section.

Table and Figure

Each table, artwork, and figure should have a clear and succinct caption (<30 words). Maps and artworks should be numbered as Fig. 1, Fig. 2, and Fig. 3 followed by a

caption (e.g, Fig. 1 Hazard maps of Kathmandu valley).

Table

Tables should be submitted as editable text without vertical and horizontal rules. Number tables consecutively following their position in the manuscript. Put footnote in the last row of the table if required.

Figures

Make sure that figure files are in an acceptable format (e.g., TIFF, JPEG, EPS, MS Office files) and with the excellent resolution (>150 dpi). Texts in figures should be of uniform lettering and sizing.

References

Please ensure that all references cited in the text are included in the reference list. Citation of a reference as 'in press' implies that the work has been accepted for publication. Unpublished works and personal communications can be mentioned in the text, but should not be listed in the reference list. References should be listed in alphabetical order.

Citation in text

1. If there is one author, include the author's last name and date of publication.
2. If there are two authors, include both authors' last names and date of publication.
3. If there are three or more authors, include the last name of the first author followed by et al. and date of publication
4. If cited in the sentence put the date of publication within a bracket after the last name
5. If cited at the end of the sentence put the last name and date within the bracket

Examples

Paudel and Heinen (2015) have argued that increasing heterogeneity in reserve may amplify biodiversity because of an increment in habitats.

Heterogeneity-based reserve selection is a novel approach that could assist in making a rapid conservation decision (Paudel and Heinen 2015).

Citations in the references

Journal articles

Crowley, T. J., 2000. Causes of climate change over the past 1000 years. Science 289:270–277.

Liczner, A. R., and C. J. Lortie, 2014. A global meta-analytic contrast of cushion-plant effects on plants and arthropods. Peer J 2:e265.

Paudel, P. K., and J. T. Heinen, 2015. Conservation planning in the Nepal Himalayas: Effectively (re) designing reserves for heterogeneous landscapes. Applied Geography 56:127–134.

Rozema, J., L. O. Björn, J. F. Bornman, A. Gaberščik, D.-P. Häder, T. Trošt, M. Germ, M. Klisch, A. Gröniger, R. P. Sinha, M. Lebert, Y.-Y. He, R. Buffoni-Hall, N. V. J. de Bakker, J. van de Staaij, and B. B. Meijkamp, 2002. The role of UV-B radiation in aquatic and terrestrial ecosystems-an experimental and functional analysis of the evolution of UVabsorbing compounds. Journal of Photochemistry and Photobiology B: Biology 66:2–12.

Book

Sokal, R. R., and F. J. Rohlf, 1995. Biometry: the principles and practice of statistics in

biological research. Third edition. W. H. Freeman, New York, New York, USA.

Book Chapter

Feeny, P., 1975. Biochemical coevolution between plants and their insect herbivores. Pages 3–19 in L. E. Gilbert and P. H. Raven, editors. Coevolution of animals and plants. University of Texas Press, Austin, Texas, USA.

Proceeding

Maiya, A. S., and T. Y. Berger-Wolf, 2010. An online sampling of high centrality individuals in social networks. Pages 91–98 in M. J. Zaki, J. X. Yu, B. Ravindran, and V. Pudi, editors. Proceedings, Advances in Knowledge Discovery and Data Mining, Part I. 14th Pacific-Asia Conference, 21–24 June 2010, Hyderabad, India. Springer, Berlin, Germany.

Thesis

LeCraw, R., 2014. The causes and consequences of functional diversity in a tropical aquatic insect community. Ph.D. Dissertation. University of British Columbia, Vancouver, British Columbia, Canada.

Copyright

nd/3.0/)that allows others to share the work with an acknowledgment of the work's authorship and initial publication in this journal.

Conflict of Interest

Requires submitting the Authors to file a conflict of interest statement with their submission.

Author(s)' Contributions

State the responsibilities performed by each author of the manuscript as follows: Conceived and designed the experiments: GB, PKP, RCP. Performed the experiments: PKP. Analyzed the data: PKP, CCP. Wrote the paper: GB, PKP, RCP.

Privacy Statement

The names and email addresses entered in this journal site will be used exclusively for the stated purposes of this journal and will not be made available for any other purpose or to any other party

List of Reviewers and Editors Vol 22(1)

Prof. Dr. Ramesh Kant Adhikari
Academician
Nepal Academy of Science and Technology (NAST)
Email: ramesh497@gmail.com

Prof. Dr. Janardan Lamichhane
Academician
Nepal Academy of Science and Technology (NAST)
Email: ljanardan@ku.edu.np

Prof. Dr. Bharat Mani Pokhrel
Academician
Nepal Academy of Science and Technology (NAST)
Email: bmp268@hotmail.com

Prof. Dr. Paras Nath Yadav
Academician
Nepal Academy of Science and Technology (NAST)
Email: pnyadav219@gmail.com

Dr. Binod Adhikari
Associate Academician
Nepal Academy of Science and Technology (NAST)
Email: binod.adhi@gmail.com

Dr. Jagat Kumar Shrestha
Associate Academician
Nepal Academy of Science and Technology (NAST)
Email: jagatshrestha@ioe.edu.np

Prof. Dr. Bhaiya Khanal
Nepal Bioheritage Forum for Resources Conservation (NBFRC)
Email: bhaiyakhanal@yahoo.com

Prof. Dr. Nilam Shrestha
Research Center for Applied Science and Technology (RECAST)
Tribhuvan University
Email: nilamspradhan@gmail.com

Prof. Dr. Rijan Bhakta Kayastha
Department of Environmental Science and Engineering, School of Science
Kathmandu University
Email: rijan@ku.edu.np

Prof. Dr.Gokarna Bahadur Motra
Institute of Engineering
Pulchowk Campus
Tribhuvan University
Email: gmotra@ioe.edu.np

Dr. Buddha Ram Shah
Chief Scientific Officer
Faculty of Science
Nepal Academy of Science and Technology (NAST)
Email: buddharshah25@gmail.com

Dr. Bimala Devkota
Chief Scientific Officer
S & T Information System Center
Nepal Academy of Science and Technology (NAST)
Email: diku98@gmail.com

Dr. Lok Ranjan Bhatta
Chief Scientific Officer
Faculty of Science
Nepal Academy of Science and Technology (NAST)
Email: lokranjan2000@yahoo.com

Dr. Deena Shrestha
Senior Scientist
Center for Health and Disease Studies Nepal
Email: deenashres@chdsnepal.org

Dr. Daya Ram Bhusal
Associate Professor
Central Department of Zoology
Tribhuvan University
Email: daya.bhusal@cdz.tu.edu.np

Dr. Marina Vaidya Shrestha
Associate Professor
Kathmandu Medical College
Kathmandu University
Email: merinavs@gmail.com

Dr. Megha Raj Banjara
Associate Professor
Central Department of Microbiology
Tribhuvan University
Email: banjaramr@gmail.com

List of Reviewers and Editors Vol 22(1)

Dr. Ananda Kumar Gautam
Retired scientist
Nepal Agricultural Research Council
Email: akg.aerd.narc@gmail.com

Dr. Jarina Joshi
Assistant Professor
Central Department of Biotechnology
Tribhuvan University
Email: jarinarjoshi@gmail.com

Dr. Shanta Pokhrel Bhattarai
Assistant Professor
Department of Chemistry
Tri-Chandra Multiple Campus
Tribhuvan University
Email: shantabhattarai2014@gmail.com

Dr. Kabi Raj Paudyal
Assistant Professor
Central Department of Geology
Tribhuvan University
Email: paudyalkabi1976@gmail.com

Dr. Anish Ghimire
Assistant Professor
Department of Environmental Science and Engineering
Kathmandu University
Email: anishghimire@ku.edu.np

Dr. Surya Kant Kalauni
Associate Professor
Central Department of Chemistry,
Tribhuvan University
Email: skkalauni@gmail.com

Mr. Jay Raj Ghimire
Deputy Director General
Department of Mines and Geology
Email: jayrajghimire@gmail.com

Ar. Kabita Pandey
Technical Officer
Faculty of Technology
Nepal Academy of Science and Technology (NAST)
kabita340@gmail.com

Mr. Chhatra Karki
Science Journalist
Nepal Forum of Science Journalists (NFSJ)
Email: chhatrakarki@gmail.com

Regd. No. 87/055/56 DAO/MOH/GON Volume 22(1) Jan-Jun 2024

Nepal Journal of Science and Technology (NJST) 22(1)
Published Year: 2024

Nepal Journal of Science and Technology (NJST)

NEPAL ACADEMY OF SCIENCE AND TECHNOLOGY (NAST)
Khumaltar, Lalitpur
GPO Box 3323, Kathmandu
Tel. 5547717, 5547715 Fax: 977-1-5547713
Email: njst@nast.org.np;scitechawareness@gmail.com

ISSN: 1994- 1412
8137 2157
e-ISSN 2382-5359 (Online)