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Research Article

BREEDING HABITAT PREFERENCE OF *Aedes aegypti* (Linnaeus, 1762) AND *Aedes albopictus* (Skuse, 1895) ALONG AN ALTITUDINAL GRADIENT IN MID-WESTERN NEPAL

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ABSTRACT
Aedes aegypti and *Aedes albopictus* are vectors of many viral diseases including dengue. They prefer different types of wet containers for breeding which differ according to season and region. Hence, their distribution range and preferred breeding containers should be known for their control. To fulfill the information gap, an intensive survey of potential larval breeding sites was conducted in different altitudinal gradients of mid-western Nepal. A total of 2056 wet containers of 11 different types in 1540 household's premises were inspected. Among them, 3.35% (n=69) containers of eight different types were positive for larvae and/or pupae of *A. aegypti* whereas 11 types of total 6.32 % (n=130) wet containers were positive for larvae and/or pupae of *A. albopictus*. Discarded tires and earthen pots were the most preferred breeding containers for *A. aegypti* and *A. albopictus* although the habitat preference was found to be different in different altitudinal gradients and seasons. The proportion of different types of available wet containers and the *Aedes* infested containers varied across the study sites and seasons. The dengue vectors show their presence up to 2438 m above sea level increasing the risk of *Aedes* borne diseases up to the high mountain region of mid-western Nepal. Hence, the extensive vector survey and change in breeding habitats either through public health education or by some form of law enforcement is essential.

Key words: *Aedes* mosquito, container preference, larval ecology, arbovirus, western Nepal

INTRODUCTION
Aedes mosquitoes are prolific invasive species around the globe (Gubler, 1998) and affect public health as they are vectors of many viral diseases such as dengue virus (DENV), Zika virus (ZIKV), Yellow fever and Chikungunya virus (CHIKV) (Lounibos, 2002).

Aedes aegypti and *Aedes albopictus* are aggressive day biting mosquito species (Pandey *et al*., 2015) and are considered as primary and secondary vectors of DENV respectively (Higa, 2011). *Aedes aegypti* is predominant urban species due to its adaptive ability to live around humans at domestic setup. This established *A. aegypti* as efficient dengue vector in urban settings even in its low density. *Aedes aegypti* is native to the Africa (Powell, 2013) and considered as invasive species for Nepal (WHO, 2006). *Aedes albopictus* was originated in tropical and sub-tropical areas of Southeast Asia region (Bonizzoni *et al*., 2013). As time passes, along with globalization of trade and travel both dengue vectors spread other regions worldwide (Kraemer *et al*., 2015) due to its strong ability to adapt in new environment (Benedict *et al*., 2007). *Aedes albopictus* is considered as native species for Nepal as it has been recorded since first mosquito survey for Nepal in 1956 (Petters & Dewar, 1956). In central and eastern Nepal, dengue vectors expand their habitat range up to altitude of 2310 m above sea level (asl) with established population (Dhimal *et al*., 2014b). Climate change, global warming, urbanization and human movement are the important components which facilitate vectors distribution and establishment in Nepal (Dhimal *et al*., 2015).

Selection of a container for egg laying by *A. aegypti* and *A. albopictus* mosquito is an important component of their life history (Bentley & Day, 1989). There is spatio-temporal variation in breeding container preference of these mosquito species. Female *A. aegypti* exploits artificial containers such as discarded tires, plastic buckets, plastic drums and other storage containers of domestic water for breeding (Focks & Alexander, 2006). *Aedes albopictus* is considered as rural species which breeds in natural containers (Darsie *et al*., 1993) such as bamboo stumps (Oli *et al*., 2024) tree holes, coconut shells etc. although it also exploits artificial containers (Barbazan *et al*., 2008). In spite the breeding preference of *A. aegypti* to the containers not used by *A. albopictus*, coexistence of both species by sharing same containers has been recorded (Kamgang *et al*., 2017).

Due to wide distribution of vectors, climate change and lack of immunity against ZIKV in the native population, there is high risk of ZIKV transmission in Nepal which may follow the footsteps of DENV which have already invaded in Nepal (Dhimal *et al*., 2018). Most of the studies were only focused on central and eastern part and the lacking from western Nepal. Hence, this research

aimed to determine the habitat preference of dengue vectors along altitudinal gradients of mid-western Nepal.

MATERIALS AND METHODS

Study area

The study area comprises five districts of Karnali and Lumbini province of mid-western Nepal (Figure 1). The Banke, Surkhet and Dailekh represent Terai, Siwalik and middle mountain regions respectively whereas Kalikot and Jumla represent high mountain region. From each study district, a single municipality was purposively selected as study site. Kohalpur municipality, Kohalpur (28°11'55" N, 81°41'28" E, 140 m asl) was selected from Banke; Birendranagar municipality, Birendranagar (28°35'48.1" N, 81°36'995" E, 705 m asl) was selected from Surkhet; Narayan municipality, Dailekh bazar (28°50'43" N, 81°42'50" E,1448 m asl) was selected from Dailekh; Manma bazar of Khadachakra municipality, (29°8'60 N, 81°37'0" E, 2033 m asl) was selected from Kalikot and Khalanga of Chhandannath municipality (29°16'55 N, 82°11'9" E, 2450 m asl) was selected from Jumla. These locations are interconnected by road webs and have population mobility. The altitude of selected study sites ranges from 140 m above sea level (asl) in the south to 2450 m asl in the North representing vertical cross-section of each physiographic region of the country except high Himalayan region.

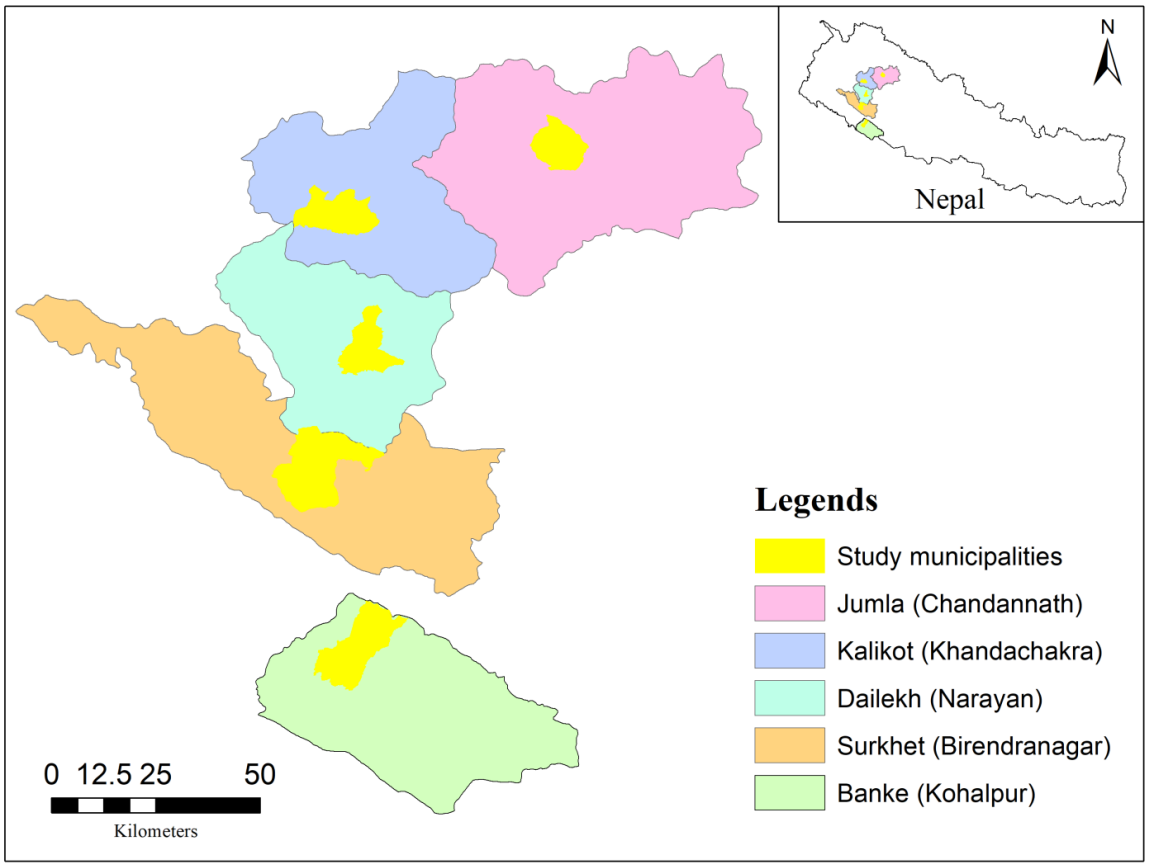

Figure 1. Map of study area (municipalities) in five districts of Nepal. The figure in the upper right box shows the location of the study area in Nepal map

Site selection and mosquito collection

Entomological survey was performed in 1540 household premises from March to November of 2022 to represent three seasons pre-monsoon (March to May), monsoon (June to August) and post-monsoon seasons (September to November). Screening of all possible *Aedes* breeding containers such as water storing drums, discarded tires, cans, beakers etc. were carried out around household premises. The mosquito larvae and pupae were collected using dropper and dipper technique from water-filled containers. Live specimens were transported to the laboratory of Surkhet Multiple Campus, Tribhuvan University, Surkhet and reared until adult emergence. The slides of dead larvae were prepared by using Hoyer's medium. The emerged adults were killed by using chloroform and preserved by carding. Larvae and adults were identified by using standard taxonomic keys developed by Darsie and Pradhan (1990) and Rueda (2004) in the laboratory of Natural History Museum, Tribhuvan University, Swoyambhu, Kathmandu, Nepal.

Data analysis

Data were arranged in MS-Excel 2010 and the container preference of dengue vectors (*Aedes aegypti* and *Aedes albopictus*) was assessed by calculating the breeding preference ratio (PBR) as follows:

X% = (Total no. of one type of container examined / Total no. of containers examined) × 100
Y% = Total no. of one type of positive container / Total no. of positive container examined) × 100
BPR (Y % / X %) = % of positive containers (Y %) / % of examined container (X %). (Gautam *et al*., 2009)

Those containers which contain water for at least three days were considered as wet containers. The inspected breeding containers in which larvae and/or pupae were present at the time of inspection were considered as infested containers. The house in which at least one *Aedes* larvae and/or pupae positive container was found was recorded as positive or infested house.

RESULTS

Altogether 2056 wet containers of 11 different types were screened for larvae and/or pupae of *Aedes aegypti* and *Aedes albopictus* in 1540 household premises. Among them, 7.97% (n=164) containers in 9.74% (n =150) households were found to be infested by *Aedes* species. Sharing of the breeding habitats between *A. aegypti* and *A. albopictus* in 1.702% (n=35) wet containers of 1.94% (n=30) households were also recorded. Other mosquito fauna recorded were *Culex* sp., *Anopheles* sp. and *Armigeres* sp. The number of houses inspected, number of containers searched, number of *A. aegypti* positive containers and number of *A. albopictus* positive containers in different study sites in different seasons are presented in table 1.

Table 1. Number of house and container scanned and number of *Aedes* positive houses and containers in study area

Study seasons and sites	No of house searched	No of container searched	No of positive house for		No of positive containers for	
			A. aegypti	*A. albopictus*	*A. aegypti*	*A. albopictus*
Pre-monsoon season						
Kohalpur	105	35	2	1	4	1
Birendranagar	104	83	1	2	1	2
Dailekh bazar	104	21	0	0	0	0
Manma	104	43	0	0	0	0
Khalanga	105	88	0	0	0	0
Total	522	270	3	3	5	3
Monsoon season						
Kohalpur	101	247	16	24	17	25
Birendranagar	103	203	11	15	11	19
Dailekh bazar	100	144	10	18	13	19
Manma	100	200	1	3	2	4
Khalanga	100	141	1	1	1	1
Total	504	935	39	61	44	68
Post-monsoon season						
Kohalpur	103	215	6	22	7	24
Birendranagar	103	159	7	15	8	15
Dailekh bazar	101	165	2	19	2	20
Manma	102	152	2	0	1	0
Khalanga	105	160	1	0	2	0
Total	514	851	18	56	20	59

Distribution of breeding containers

The number of different types of potential breeding containers for *Aedes* species varied according to study sites and seasons in which plastic drums were most abundant potential breeding container in pre-monsoon and monsoon season whereas discarded tires were in post-monsoon season. The proportion of different types of breeding containers significantly varied across the study sites in pre-monsoon ($\chi^2 = 266.26$, $p < 0.001$), monsoon ($\chi^2 = 60.09$, $p = 0.021$) and post-monsoon ($\chi^2 = 117.6$, $p < 0.001$) seasons. Although all types of *Aedes* breeding containers were available from lowland terai to high mountain region, *A.aegypti* and *A. albopictus* infested particular type of wet containers in different altitudinal ranges (Figure 2).

Breeding preference ratio (BPR) of *Aedes aegypti*

Breeding preference ratio (BPR) depicts the breeding habitat preference which varied according study site in pre-monsoon, monsoon and post-monsoon seasons. During survey period, discarded tires were most preferred breeding containers for *A. aegypti* from terai to middle mountain region whereas wooden structure for animal feed and plastic drums respectively were most preferred *A. aegypti* breeding containers in Kalikot and Jumla of high mountain regions (Table 2).

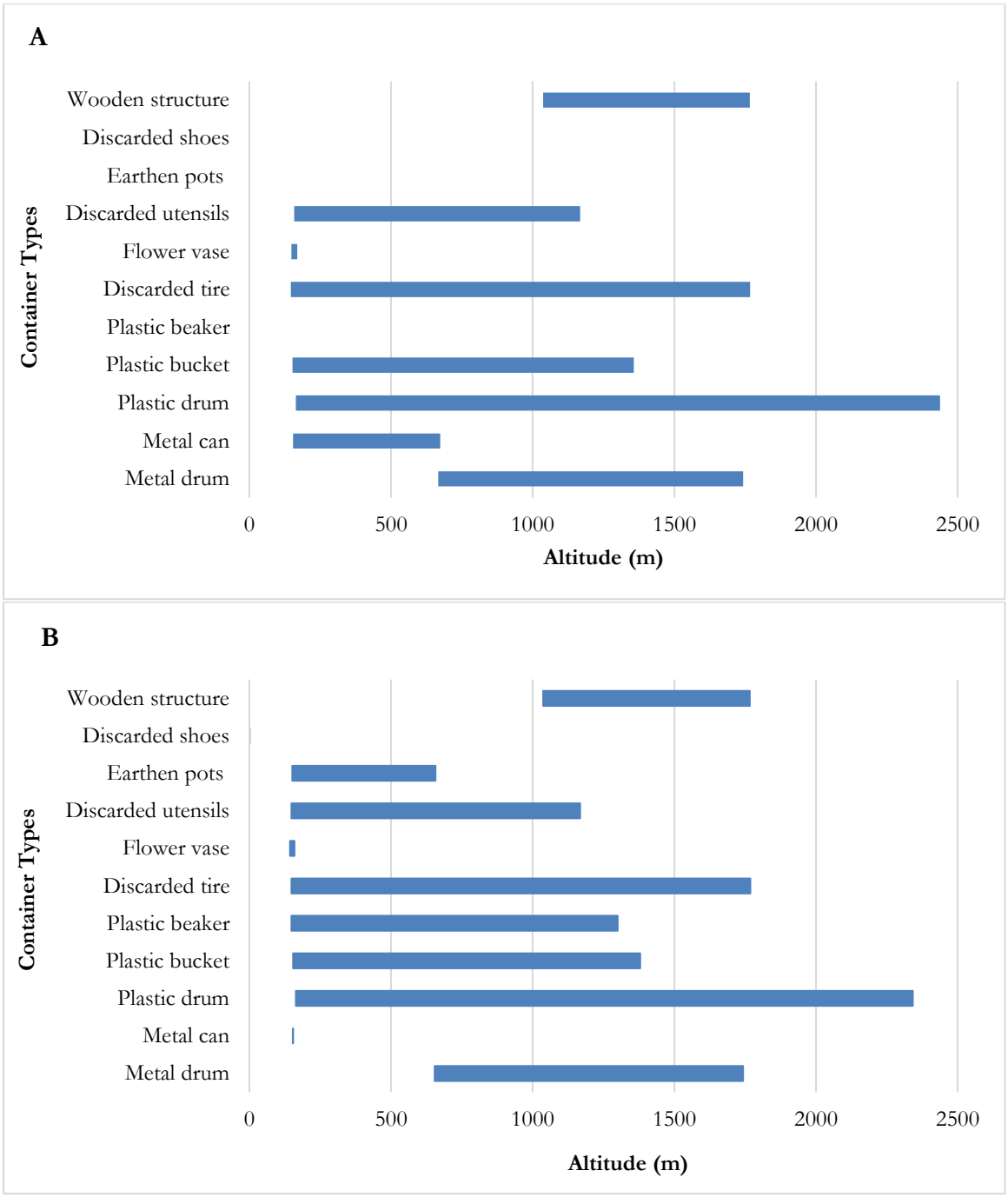

Figure 2. Distribution of A. *Aedes aegypti* and B. *Aedes albopictus* infested containers along altitudinal gradients of mid-western Nepal.

The BPR for *A. aegypti* was also calculated for each study location in each season to know spatial variation in habitat preference in different seasons. The BPR was found to be highest for discarded tire in Kohalpur in all seasons. Similarly, plastic drums (BPR = 1.97) in pre-monsoon, discarded tires (BPR = 1.99) in monsoon and metal drums (BPR = 3.31) in post-monsoon season were highly preferred breeding containers in Birendranagar. Likewise, in Dailekh bazar, the highest BPR was recorded from discarded tires (BPR = 6.04) in monsoon and metal drums (BPR = 6.85) in post-monsoon season. No larvae and/or pupae of *A. aegypti* were recorded in pre-monsoon season from Dailekh bazar, Manma and Khalanga. In Manma, the BPR was found to be highest for metal drum (BPR = 8.33) in monsoon and discarded utensils (BPR = 8.33) in post-monsoon season whereas the BPR was highest for plastic drums in Khalanga in monsoon and in post-monsoon season (Table 2).

Table 2. Breeding preference ratio (BPR) of *Aedes aegypti* in study area

Seasons and study sites	Breeding preference ratio (BPR) of *A. aegypti*										
	Metal drum	Metal can	Plastic drum	Plastic bucket	Plastic beaker	Discarded tire	Flower vase	Discarded utensils	Earthen pot	Discarded shoes	Wooden structure
Pre-monsoon											
Kohalpur	0	0	0	0	0	**3.88**	0	0	0	0	0
Birendranagar	0	0	**1.97**	0	0	0	0	0	0	0	0
Dailekh bazar	0	0	0	0	0	0	0	0	0	0	0
Manma	0	0	0	0	0	0	0	0	0	0	0
Khalanga	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	0.40	0	0	**7.17**	0	0	0	0	0
Monsoon											
Kohalpur	0	0	0.28	0.44	0	**3.96**	0.78	0.19	0	0	0
Birendranagar	1.84	1.41	1.50	0	0	**1.99**	0	0	0	0	0
Dailekh bazar	1.84	0	0.79	1.44	0	**6.04**	0	0	0	0	25
Manma	**8.33**	0	0	0	0	0	0	0	0	0	0
Khalanga	0	0	**3.71**	0	0	0	0	0	0	0	0
Total	1.39	0.36	0.69	0.52	0	**3.92**	0.45	0.17	0	0	1.93
Post-monsoon											
Kohalpur	0	2.55	0	0.93	0	**4.55**	0	0.68	0	0	0
Birendranagar	**3.31**	0	1.24	0	0	1.98	0	0	0	0	0
Dailekh bazar	**6.85**	0	0	0	0	0	0	5.15	0	0	0
Manma	0	0	0	0	0	0	0	**8.33**	0	0	0
Khalanga	0	0	**4.21**	0	0	0	0	0	0	0	0
Total	2.07	0.67	1.19	0.31	0	**2.25**	0	0.29	0	0	0
During total survey period (including all seasons)											
Kohalpur	0	0.52	0.28	0.51	0	**4.43**	0.48	0.16	0	0	0
Birendranagar	2.02	0.63	1.26	0	0	**2.15**	0	0	0	0	0
Dailekh bazar	2.12	0	0.66	1.65	0	**2.69**	0	0.12	0	0	0
Manma	**3.87**	0	0	0	0	0	0	0.15	0	0	18.80
Khalanga	0	0	**2.86**	0	0	0	0	0	0	0	0

Breeding preference ratio (BPR) of *Aedes albopictus*

In total survey period, different containers were found to be preferred by *A. albopictus* to breed in different study sites. The BPR of *A. albopictus* was highest for earthen pots in pre-monsoon season and for discarded tires in monsoon and post-monsoon season. The discarded tires and earthen pots were the most preferred breeding container for *A. albopictus* in terai and Siwalik range whereas wooden structure for animal feed has highest BPR in middle mountain region and Manma of high mountain region. The plastic drums were the most-preferred breeding container for *A. albopictus* from Jumla district of high mountain region (Table 3).

Table 3. Breeding preference ratio (BPR) of *Aedes albopictus* in study area

Seasons and study sites	Breeding preference ratio (BPR) of *A. albopictus*										
	Metal drum	Metal can	Plastic drum	Plastic bucket	Plastic beaker	Discarded tire	Flower vase	Discarded utensils	Earthen pot	Discarded shoes	Wooden structure
Pre-monsoon											
Kohalpur	0	0	0	0	0	0	0	**8.75**	0	0	0
Birendranagar	0	0	0	0	0	0	0	0	**27.66**	0	0
Dailekh bazar	0	0	0	0	0	0	0	0	0	0	0
Manma	0	0	0	0	0	0	0	0	0	0	0
Khalanga	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0.33	**60**	0	0
Monsoon											
Kohalpur	0	0	0.19	0.59	0	**3.14**	0.80	0.57	1.41	0	0
Birendranagar	2.13	0	0.43	0	1.88	**2.59**	0	0	0.89	0	0
Dailekh bazar	0.63	0	1.35	1.97	0.44	**4.13**	0	0	0	0	0
Manma	**4.16**	0	0.62	0	0	3.12	0	0	0	0	12.5
Khalanga	0	0	**3.71**	0	0	0	0	0	0	0	0
Total	1.08	0	0.55	0.68	0.68	**3.46**	0.44	0.52	0.91	0	0
Post-monsoon											
Kohalpur	0	1.49	0	1.08	1.17	**1.65**	1.11	0.59	1.27	0	0
Birendranagar	1.76	0	0.33	0	0.66	1.27	0	0.28	**5.3**	0	0
Dailekh bazar	1.37	0	0.88	0.48	**1.87**	1.45	0	1.03	0	0	0
Manma	0	0	0	0	0	0	0	0	0	0	0
Khalanga	0	0	0	0	0	0	0	0	0	0	0
Total	1.05	0.45	0.40	0.53	1.36	**1.44**	1.22	0.67	3	0	0.96
During total survey period (including all seasons)											
Kohalpur	0	0.58	0.15	0.86	0.82	**2.36**	0.95	0.56	1.42	0	0
Birendranagar	1.87	0	0.30	0	1.23	1.99	0	0.40	**3.76**	0	0
Dailekh bazar	0.81	0	1.02	1.48	1.30	2.07	0	0.25	0	0	**2.11**
Manma	2.90	0	0.74	0	0	2.05	0	0	0	0	**14.10**
Khalanga	0	0	**2.86**	0	0	0	0	0	0	0	0

In pre-monsoon season, discarded utensils (BPR = 8.75) and earthen pots (BPR = 27.66) were most preferred breeding container for *A. albopictus* in Kohalpur and Birendranagar respectively. As no larvae of *A. albopictus* collected from other study sites in pre-monsoon season, the BPR was not assessed. In monsoon season, *A. albopictus* preferred discarded tires in Kohalpur (BPR = 3.14), Birendranagar (BPR = 2.59) and Dailekh bazar (BPR = 4.13) but preferred metal drum (BPR = 4.16) and plastic drum (BPR = 3.71) in Manma and Khalanga respectively. The BPR of *A. albopictus* was highest for discarded tire in Kohalpur (BPR = 1.65), earthen pots in Birendranagar (BPR = 5.3) and plastic beaker (BPR = 1.87) in Dailekh bazar. From Manma and Khalanga, no any larvae and/or pupae of *A. albopictus* were recorded in post-monsoon seasons (Table 3).,

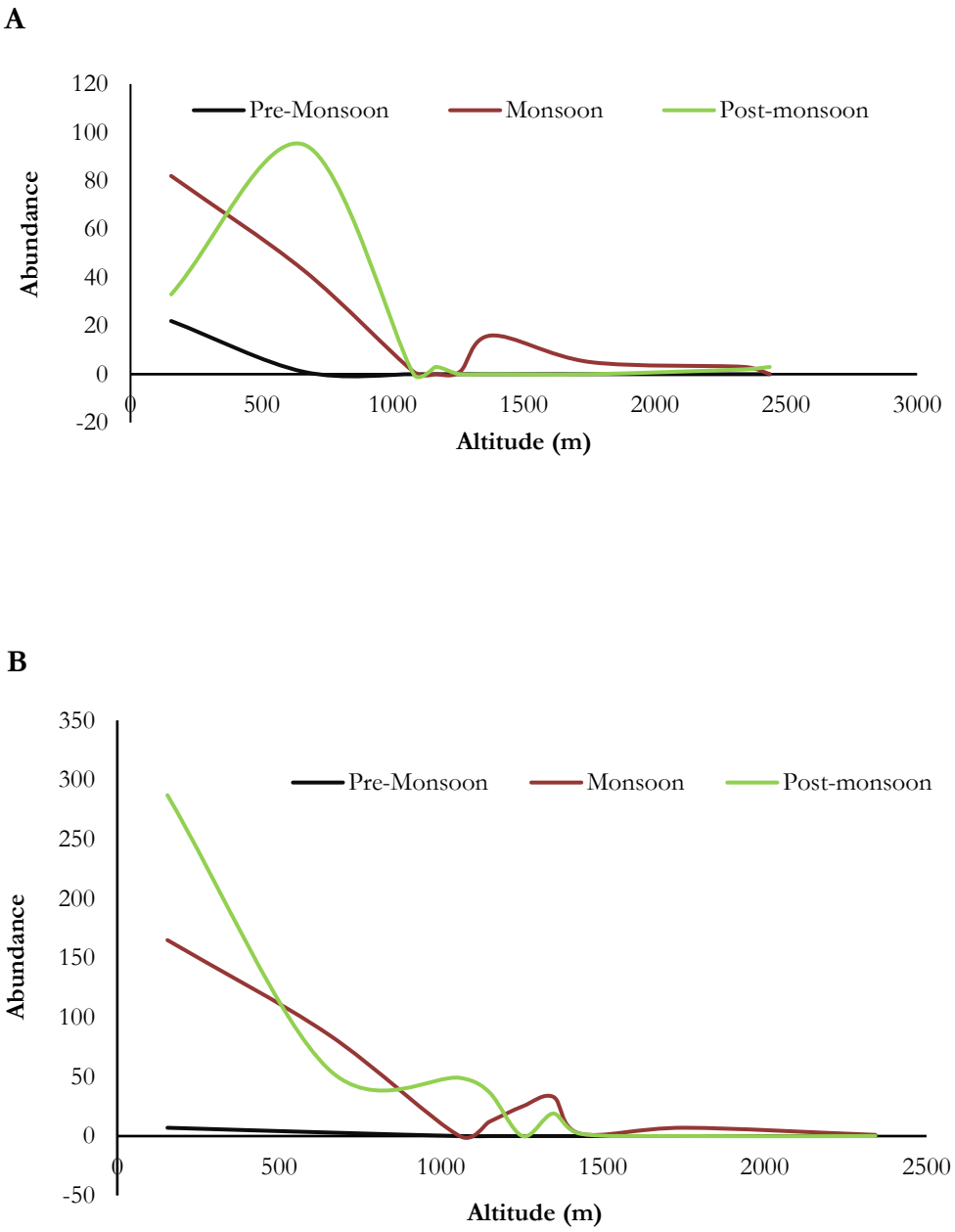

Figure 3. Seasonal abundance of **A**. *Aedes aegypti* and **B**. *Aedes albopictus* in different altitudinal gradients (**A**. *Aedes aegypti* and) in mid-western Nepal.

Altitudinal distribution *Aedes aegypti* and *Aedes albopictus*

Aedes aegypti and *Aedes albopictus* were recorded from all altitudinal ranges. The larvae and/or pupae of *A. aegypti* was recorded from 146 m asl of lowland terai (Banke) to 2438 m asl of high mountain region (Jumla) whereas the larvae and/or pupae of *A. albopictus* were recorded in all study locations from 143 m asl of lowland terai (Banke) to 2342 m asl in high mountain regions (Jumla). The abundance of *A. aegypti* declined with increasing altitude in pre-monsoon and monsoon season but in post-monsoon season, the *A. aegypti* population increased up to Swalik range and then sharply declined with increasing altitude. The larvae and/or pupae of *A. albopictus* were abundant from lowland terai to middle mountain region with declining population in all three seasons and show their existence in high mountain region (Manma and Khalanga) only in monsoon season (Figure 3

DISCUSSION

In the present study, *Aedes aegypti* and *Aedes albopictus* were recorded in a variety of peri-domestic breeding containers (Fontenille & Toto, 2001). Among 11 different types of examined water holding containers, *A. aegypti* infested eight different types of containers whereas *A. albopicts* infested all 11 types of containers. The infestation of more types of breeding containers by the *A. albopictus* indicates its wide range of breeding habitat preference and more adaptive capacity (Kraemer *et al*., 2015). The breeding habitat sharing of *A. aegypti* and *A. albopictus* has been recorded (Braks, 2003; Hashim, 2018) which suggest convergent habitat segregation for both species.

Site-wise and season-wise variations in number of dengue vectors breeding containers were recorded due to variations in temperature and rate of precipitation (Gautam *et al*., 2012). In monsoon season, a number of discarded containers lying outdoor get filled with rainwater and became possible breeding containers for dengue vectors. Subsequently, low rainfall in post-monsoon season reduces the number of water-holding containers which ultimately declined the population of larvae. Due to high temperature and low rainfall in pre-monsoon season, number of water holding containers decreases resulting in a low population of immature *Aedes* species (Wijayanti *et al*., 2016). Among infested containers, discarded tires were the most preferred breeding container for *A. aegypti* in all three seasons as recorded by Mudin (2015).

The BPR was high for *A. aegypti* in discarded tires in Kohalpur, Birendranagar and Dailekh bazar whereas it's high in wooden structure for animal feed in Manma and plastic drum in Khalanga. Kohalpur, Birendranagar and Dailekh bazar have more urban settings where garages are established for reconditioning of vehicles. People piled up the discarded tires with the hope to re-use or recycle in the future. In Manma bazar, there is proper drinking water facility and in associated villages, peoples use wooden structures for animal feed but in Khalanga (Jumla) there is poor water supply and people store water in plastic drums and those drums without lids are never get empty are the breeding habitats for *Aedes* mosquitoes.

People use earthen pots for water storage as well as in local technology of fermentation and distillation process in peri-urban areas by Tharu community. The breeding preference of *A. albopictus* to the earthen pots as breeding container may be its behavior to prefer natural containers although it differs in different seasons and sites. At the monsoon season, the discarded tires also accumulate rain water which get heated quickly and act as most preferred breeding container for *A. albopictus* too. The BPR was high for discarded tire in Kohalpur as it is urban area and has number of garages for the vehicle services. This provides a lot of discarded tires which are kept piled up for long time, remain undisturbed and act as possible breeding containers for *A. albopictus*. Likewise, *A. albopictus* utilizes wooden structure for breeding in Dailekh Bazar and Manma but the BPR was higher in plastic drum in Khalanga, Jumla. Because people use the wooden structures for animal feed in villages of the Dailekh Bazar and Manma and plastic drum in Khalanga, Jumla.

The present entomological survey recorded dengue vectors from lowland districts of terai region to the high mountain region in mid-western Nepal. This finding coincides with the study conducted in eastern Nepal (Dhimal *et al*., 2015). We recorded a high abundance of larvae and/or pupae of dengue vectors up to middle mountain region. The established population of dengue virus vectors has been recorded up to middle mountain region of Nepal by different studies (Gautam *et al.,* 2009; Gautam *et al*., 2012; Dhimal *et al*., 2014a); that may due to warm climatic conditions in lower altitudes (Bangs *et al*., 2006). The geographical and habitat expansion of *A. aegypti* and *A. albopictus* up to the high mountain region may be due to transportation facilities, human movement, establishment of new urban areas, improper solid waste management and climate change as a result of global warming. This may be supported by the presence of possible breeding habitats, poor water supply, unplanned urbanization and lack of public health awareness (Vitek *et al*., 2014).

CONCLUSIONS

In mid-western Nepal, *A. aegypti* and *A. albopictus* have well established population up to middle mountain region and show their presence in high mountain region. They exploit artificial discarded containers for breeding. The *A. aegypti* prefer discarded tires and *A. albopictus* prefer earthen pots for breeding. The breeding preference of *A. aegypti* and *A. albopictus* differs according to season and study site. Hence, public awareness programs along with extensive vector surveillance are essential to improve dengue vector controlling interventions in mid-western Nepal.

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AUTHORS CONTRIBUTION

BRO and PS: Field data collection; BRO, MS, and IG: rearing and preservation of specimens; BRO, PS, and IG: identification of mosquito specimens; BRO, MS, and IG: data analysis and writing of original draft; BRO and IG: research design; IG and MD: validation, consultation, writing revision and editing the manuscript. All the authors have read and agreed for publishing the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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