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A CROSS-SECTIONAL SURVEY OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IMMATURE IN THE DISCARDED TIRES

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ABSTRACT

Vector surveillances are done periodically, to know the prevalent mosquito in a locality, to predict the outbreak of mosquito-borne diseases, to derive effective vector control strategies, etc. Surveillance of different containers would help to set the threshold level of stegomyia indices to predict the outbreak and infestation level. This study aims to determine the infestation level of dengue vectors *Aedes aegypti* and *Aedes albopictus* in discarded tires during June and July 2022. A survey of larvae and pupae in the discarded tires of automobile workshop, of two sites Manohara corridor Sadak and Kalanki was carried out. Stegomyia indices, Container index (CI), and House index (HI) were calculated. The emerged adult mosquitoes were identified morphologically after rearing the pupa and larvae in a plastic cup.

In June and July 2022, 11 automobile workshops were visited to inspect the presence of larvae and pupae in the discarded tires. Of the total 44 tires inspected, 11 tires were positive for larvae and pupae. Therefore, the Container index (CI) percentage was 25% and the House index was 36.36%. Of the 410 larvae and pupae reared *Aedes aegypti* 73.87%, *Aedes albopictus* 13.48%, and other 12.64% were present in the discarded tires. *Ae. albopictus* was present only in the discarded tires of the Kalanki site. The dominant vector was *Ae. aegypti*. The discarded tires are shared as breeding habitat by *Ae. aegypti* and *Ae. albopictus*. CI and HI percentages predict the risk of dengue outbreaks in the future.

Keywords: Aedes aegypti, Aedes albopictus, dengue, surveillance, tires

INTRODUCTION

Aedes aegypti and *Aedes albopictus* are vectors for more than one arboviral disease. *Ae. aegypti* native to Africa, and *Ae. albopictus* indigenous to Asia, were wild species, that have adapted to breed in the artificial containers of rural, suburban, and urban human environments. (WHO, 2011). They are introduced to the rest of the nation by global trade and travel (Devi *et al.*, 2020; Kraemer *et al.*, 2015; WHO, 2011). The extending expansion of *Aedes* in Nepal is a threat to Zika, chikungunya, and other arboviral infections (Dhimal *et al.*, 2018; Poudel, 2022).

Periodical larva and pupa surveillance in different containers are necessary to monitor infestation levels (WHO, 2003, 2011). Stegomyia indices House Index (HI) >5% or Breteau Index (BI)>20 indicate risk of dengue outbreak (Liyanage *et al.*, 2022; Sanchez *et al.*, 2006; WHO, 2003). National guidelines of Nepal also recommend HI, CI, and BI to record the *Ae. aegypti* infestation level (EDCD, 2019).

The research aims to compute stegomyia indices Container index (CI%) and House index (HI%) of the discarded tires in automobile workshops of Manohara corridor Sadak near Narephat Koteshwor and Kalanki resoling garage, Kathmandu in June and July 2022 and to identify *Ae. aegypti* and *Ae. albopictus*.

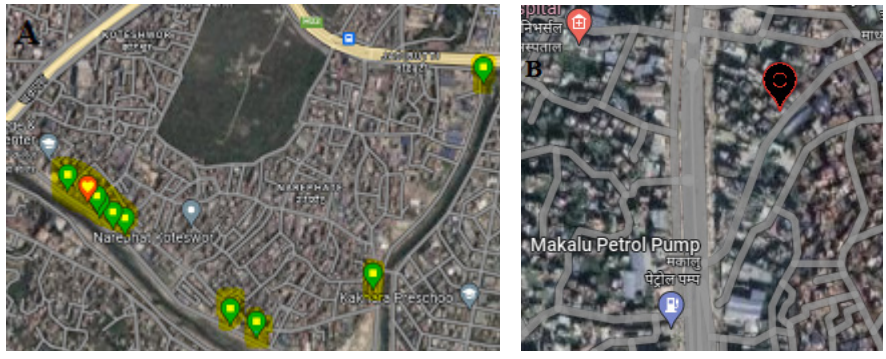
METHODS AND MATERIALS

A cross-sectional survey was carried from June-July 2022 at two sites (i) Manohara corridor Sadak (ii) Tire resoling garage of Kalanki for an entomological survey of larva and pupa in the discarded tires of automobile workshop to enumerate stegomyia indices CI% and HI% of the discarded tires in automobile workshops, and to assess the presence or absence of *Ae. aegypti* and *Ae. albopictus* at these sites. Ethical approval was granted by Nepal Health Research Council to carry out the research work (Registration 79/2020). These two sites are selected as the sampling sites, as they are the major entry points of vehicles from various districts of Nepal to Kathmandu as well as entry of goods carrying trucks from neighboring nations. The number of automobile workshops and the discarded tires were taken as the sample size. Manohara corridor Sadak extending from Jadibuti to Balkumari Bridge was selected. Manohara corridor Sadak is surrounded by Bhaktapur and Lalitpur districts at the east, Kathmandu district at the northwest, and Lalitpur in the south. The river divides the three districts

Lalitpur, Bhaktapur, and Kathmandu (Figure 1A). This locality includes a large number of automobile workshops and garages.

Figure 1

Satellite picture of the two sites chosen for larva and pupa collection. A. Manohara corridor Sadak (27.6713 ^{0}N, 85.3474^{0}E). B. Kalanki (27.6931^{0}N, 85.3474^{0}E). The visited field area is highlighted in both maps. The picture appears on the following websites. www.Manohara+Corridor+Sadak,+44600/@27.6766251,85.2979346,13.94z/,www.Kalanki/@27.6981387,85.2803193,17z/



Only one automobile workshop was included during the study in Kalanki comparatively bigger tire resoling center (Figure 1B). The GPS coordinates are being added to locate the exact place on the map. From these sites' CI % was calculated by using the formula.

CI = {No. of positive containers / No: of containers inspected} X100. Similarly, (HI or Premises Index (PI) was calculated by using the formula HI= {No. of infested automobile workshops /No. of automobile workshops inspected} X100. (WHO, 2011). Considering the households as automobile workshops, where unwanted tires are discarded and serve as a breeding habitat after retaining rainwater during the rainy season.

Discarded tires in the above-mentioned site were inspected for larvae and pupae. Larvae and pupae were collected from the discarded tires by aspirating with a help of a dropper, dipping, and ladle (EDCD, 2019). In the case of tires with turbid water absence or presence of immature was inspected by placing the water in transparent plastic cups. Larvae and pupae were transported to the laboratory in a zip-lock bag containing clean water. Twenty-five larvae from the positive container were separately placed in

a transparent plastic cup until adult emergence. The larvae were fed by adding a pinch of dog biscuit and bakery's yeast (Devidas *et al.*, 2014).

Figure 2

Tires used for collection of larvae and pupa from the two sites. A. Tires of Kalanki automobile workshop. B. Tires of Manohara corridor Sadak workshop.

Similarly, 10 pupae from each positive tire were kept in a plastic cup until the emergence of an adult mosquito (Figure 3B). All the 25 larvae and 10 pupae after adult emergence were identified and the percentage of *Ae. aegypti* and *Ae. albopictus* present in the tires were determined.

Figure 3

Laboratory rearing of collected pupae and larvae. A. Cup containing larvae for rearing with larval food. B. Cups containing pupa covered with muslin cloth to avoid the escape of emerged adult mosquitoes.

The emerged adult mosquitoes were placed in a test tube by using an aspirator, with the help of a magnifying lens and identification keys,

identified as *Ae. aegypti* and *Ae. albopictus* to genera, species, and sex (Rueda, 2004; WHO, 2020). Other types of mosquitoes were not included in the study.

The data collected were transferred to a Microsoft Excel file with respective codes and then CI and HI indices were determined. The percentage of *Aedes* presence and their female and male ratio were calculated manually.

RESULTS AND DISCUSSION

At the Manohara site, ten automobile workshops (AWK1-10) were visited three workshops (AWK5, AWK5, and AWK9), were positive for larvae and pupae. A total of six tires were positive for larva and pupa at this site (Table 1). Therefore, the container index percentage (CI %) was 15.38%. In the case of Kalanki (KN1), all five tires inspected contain immature larvae and pupae (Table 1). The CI in the case of the Kalanki site is 100%. The total number of tires inspected from the two sites was 44 and the positive tires are 11 therefore CI was 25%. HI index was calculated as 36.36% from the two sites. Large numbers of tires of different sizes not managed properly thrown outside in an open space collect rainwater during the rainfall and serve as a breeding habitat for mosquitoes (WHO, 2003). In general, 25% {(11/44) X100} of tires are infested and 40.90% {(18/44) X100} of the tires contain water. The percentage of water holding tires infested with larvae and pupae was 25%, i.e., CI is 25% of discarded tires containing water are infested with larvae and pupae. HI was 36.36% which indicates 36.36% of automobile workshops are infested. The CI was 15.38% in the Manohara site and 100% in the Kalanki site. In the Manohara site in some of the workshops, the discarded tires were inside the shed and no accumulation of rainwater was found during the visit so devoid of breeding habitat for mosquitoes. In some of the workshops, the tires contain water as well as mobil oil or grease in it that prevented the breeding habitat. In some tires water was present but no larvae or pupae was detected. The absence of larvae and pupae was confirmed by emptying the water from the tires and also by observing the absence of immature by placing the water in a transparent plastic cup. So, not all the tires inspected were positive for larvae and pupa in the Manohara site. Catchment of water in the tires is the main reason to increase the infestation level. The tires left undisturbed for a long duration in the open place collect rainwater and help to proliferate the mosquito genera. The results show not only *Aedes* spp. breed in the tires other genera are also able to breed in the discarded tires. In the case of Kalanki the CI was 100%, all the inspected tires were positive for larva and pupa all the tires were in the open field.

CI of the two sites is 25%, which indicates that discarded tires provide an excellent breeding habitat and a higher infestation rate. The high larval indices CI 25% and HI 36.36% may be due to the first rain in June favorable for the breeding of *Aedes* spp. the result is in agreement as observed (Chaikoolvatana *et al.*, 2007; Oo *et al.*, 2020). The entomological tools currently in use for assessing this risk of dengue outbreak are the stegomyia indices CI, HI, and BI (Bowman *et al.*, 2014; WHO, 2009). During the year 2022 there was a massive outbreak of dengue in Nepal, recording a total number of 53951 cases as of 30th NOV 2022 (EDCD), 2022). Thus, CI index reflects the risk of dengue, HI 36.36% and CI 25% should be considered as an indicator of dengue outbreak as these indices are obtained from a single type of container (discarded tires). A similar result was obtained in a study as discarded tires as a key container, CI 28.36%, and HI 37.7% resulted an outbreak of dengue in Tamil Nadu, India (Bajwala *et al.*, 2022). Comparing the stegomyia indices CI >20% and HI> 35% the risk of an epidemic is high (Padonou *et al.*, 2020). The CI and HI indices of the results indicated the risk of a dengue outbreak. It has become necessary to monitor the indices regularly for assessing the risk of vector-borne diseases, due to climate changes the vectors are spreading from endemic regions to non-endemic regions.

Table 1

Number of discarded tires positive for larva and pupa at Manohara and Kalanki site.

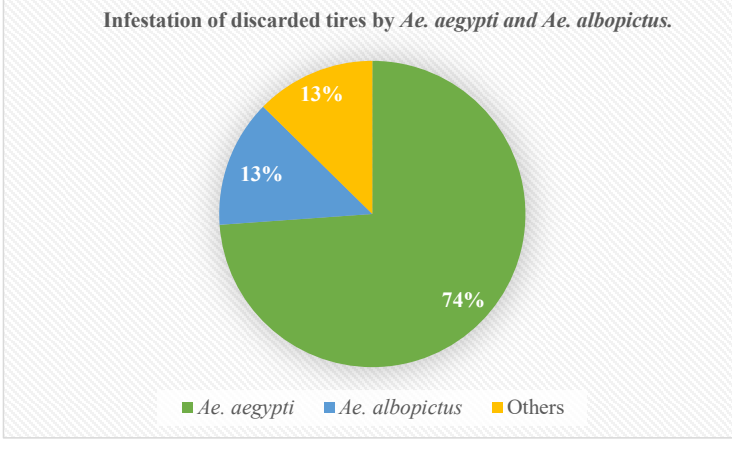
S.No.	Workshop code	No. of discarded tires inspected	No. of Discarded tires with water	No. of tires with Larva/ Pupa
1	AWK1	4	0	0
2	AWK2	3	3	0
3	AWK3	7	2	0
4	AWK4	4	4	4
5	AWK5	2	2	1
6	AWK6	7	1	0
7	AWK7	3	0	0
8	AWK8	3	0	0
9	AWK9	4	1	1
10	AWK10	2	0	0
11	KN1	5	5	5
Total	11	44	18	11 tires positive for larva and pupa

Ae. albopictus were not found (0%) from the larvae and pupae reared in the laboratory collected from the Manohara corridor Sadak site. *Ae. albopictus* were only present in samples collected from the Kalanki site. The dominant vector from both sites was *Ae. aegypti*. From the total larvae and pupae reared in the laboratory 86.82% of adult mosquitoes emerged successfully, 73.87% of *Ae. aegypti*, 13.48% were *Ae. albopictus* and 12.64% were other types of mosquitoes present in the discarded tires (Figure 4). Pupa collected from the discarded tires had a 100% capacity to develop into an adult mosquito. *Ae. albopictus* were not detected in the Manohara site may be due to insufficient rearing of collected samples or as the site may not be favorable for breeding. *Ae. albopictus* as it tends to occur more commonly in areas with open spaces and vegetation (Camargo *et al.*, 2021; Champion & Vitek, 2014; WHO, 2011). Manohara site lacks vegetation and it is a polluted river basin so that may be the reason for the absence of *Ae. albopictus*.

Ae. albopictus were only present in the samples collected from the Kalanki site. Kalanki automobile workshop deals with the work of truck tires so, that may be the reason for the presence of *Ae. albopictus*. Big tires are the source of *Ae. albopictus* eggs transported due to trade (WHO, 2011). As well as the backyard of the garage a small field had a lot of vegetation like maize, beans, trees, bushes, etc. the vegetation supported *Ae. albopictus*. A mixed infestation was observed in the discarded tires of the Kalanki site.

Figure 4

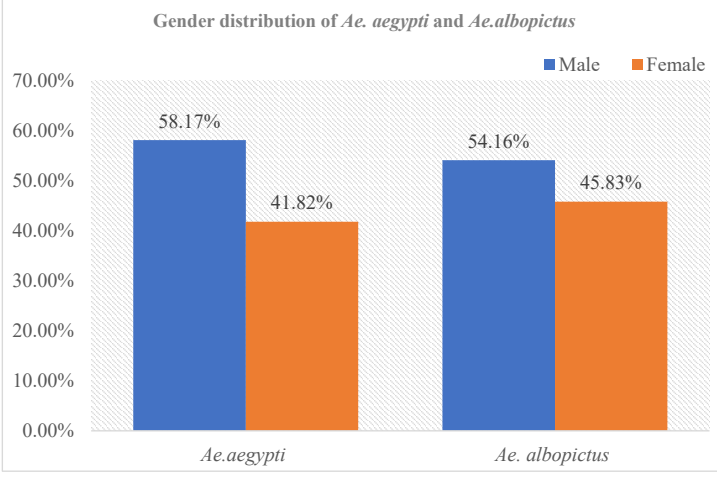
Infestation of discarded tires by Ae. aegypti and Ae. albopictus.



Male mosquitoes were dominant compared to the female during the short-term study period (Figure 5). *Ae. aegypti* male and female were 58.17%and 41.82% respectively. *Ae. albopictus* male and female were 54.16% and 45.83% respectively. Emerged adults were 82% from the larvae and 100% from the pupa. Only 12.64 % were other types of mosquito larvae present in the discarded tires. This proves that tires are the main breeding habitat of *Aedes* spp. Most of the study has shown the greater density of *Aedes* breeding in discarded tires (Banerjee *et al.*, 2015; Devi *et al.*, 2020; Gautam *et al.*, 2012). From the total emerged adult mosquitoes *Ae. aegypti* were 73.87% and *Ae. albopictus* was 13.48% thus *Ae. aegypti* is the dominant species breeding in the discarded. This was similar to the results obtained as 88.5% *Ae. aegypti* in a study (Devi *et al.*, 2020). Only 18% of larval deaths occurred while rearing in the laboratory condition.

Figure 5

Gender distribution of Ae. aegypti and Ae. albopictus in discarded tires.

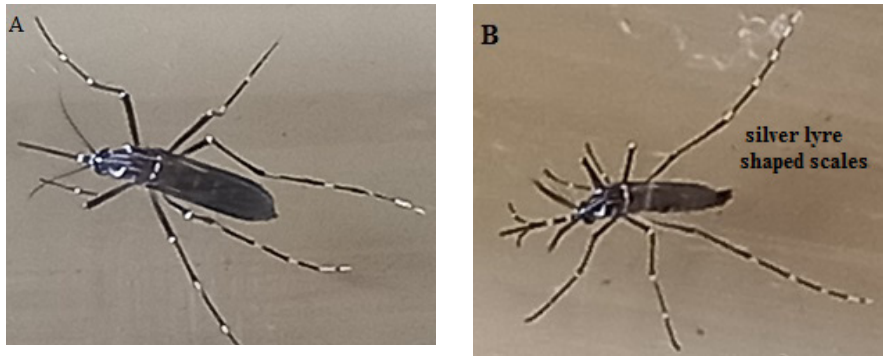


The identification of mosquito species is essential for surveillance, to know the distribution of species, to know the breeding habitat of the species, and for vector control during the high epidemic season or region (EDCD., 2019; Rueda, 2004; WHO, 2003, 2020). Male mosquitoes were identified by observing feathery antennae and palpi longer than the proboscis. Females were identified by observing less hairy antennae and palpi ¼ to half of the proboscis as described (ECDC, 2022; WHO, 2020). The key distinguishing feature observed for *Ae. aegypti* and *Ae. albopictus* were dorsal parts of the thorax with silver scales in the shape of a lyre

or appear as the half-moon in both males and females as *Ae. aegypti* and dorsal part of the thorax with a median silver single scale line in both males and females as *Ae. albopictus* (ECDC, 2022; WHO, 2020).

Figure 6

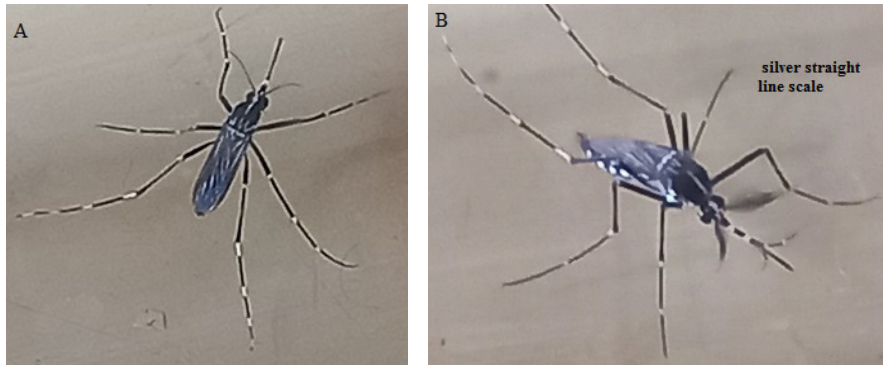
Image of Aedes aegypti. A. Female B. Male.



Male *Aedes* species were dominant from the reared larvae and pupa. Male domination may be as the sample was collected once and randomly selected larvae and pupae were reared not all the collected larvae and pupa were reared in the laboratory so, the result shows the male dominance. The result is consistence with the result of Camargo *et al.*, (2021) the reason for male dominance may also be the female mosquito may have developed faster than the male, so a greater number of male larvae was present in the container or as the female require blood as a protein source to lay eggs after emergence flied in search of blood. As in the case of pupa

Figure 7

Image of Ae. albopictus. A. Female. B. Male.



rearing male and female percentage is quite closer than in the case of larvae this proved that male and female *Aedes* present in the discarded tires may be equal in ratio or varies during the season and also depend upon the distribution of food sources.

In the context of Nepal, no standard value for dengue vector indices (HI, CI, and BI) has been set to predict the risk of disease. Larval indices HI or PI, BI, and CI the threshold values of these indices are the requirement for the vector control program as well as to predict the disease outbreak. Data collected from 11 workshops predict the CI and HI, for BI determination 100 households should be visited or 100 automobile workshops should be visited. This pilot study couldn't include 100 workshops to calculate BI index. Rearing of all the collected larvae and pupa may provide an actual ratio of male and female *Aedes* in the discarded tires. Regular surveillance, monitoring, and management of discarded tires is necessary to prevent the multiplication of dengue vector.

CONCLUSION

The discarded tires served as breeding habitats for both *Ae. aegypti* and *Ae. albopictus*. *Ae. aegypti* being the dominant species. In case of Kathmandu increment in vehicles has generated to establish the automobile workshops in the residential areas. The tires serve as unnoticed containers which helps in proliferation of *Aedes* genera. As well as the color of the tires black, an excellent oviposition for the *Aedes*. The CI and HI indices indicate requirement for regular monitoring and create awareness among the owners for management of tires. The threshold value for HI, BI, and CI should be determined by longitudinal study of regular surveillance of mosquito vectors in discarded tires. Periodical monitoring of the tires and management of the tires prevents arboviral infection.

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