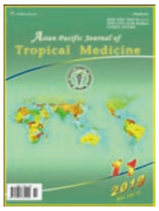

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Efficacy of diurnal BG−Sentinel traps to capture nocturnal adult *Armigeres subalbatus* mosquitoes and impact of altitudinal variations in forests

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ABSTRACT

Objective: To establish an efficacious and efficient surveillance method of *Armigeres (Ar.) subalbatus*, a known filarial vector, surviving in forest habitats to estimate realistic population density and assess the impact of altitudinal variations on the efficacy of the trap.

Methods: In the study, 12 locations in areas with an altitudinal range from 82 m to 920 m were selected in three reserve forests for night sampling of adult *Ar. subalbatus* mosquito using standard chemical lure based BG-Sentinel traps and CDC-light traps in pairs. Effects of locations and time were estimated on the efficacy of traps as mosquito density using multifactor analysis of variance for significant differences. Impact of altitudinal variations on the efficacy of traps was assessed using multiple regression with slope comparison.

Results: BG-Sentinel trap collected significantly more adult *Ar. subalbatus*, 4.43 folds in Gorumara NP, 5.19 folds in Neora Valley NP and 12.10 folds in Mahananda WLS than the CDC-light trap irrespective to locations. BG-Sentinel traps were tolerant of altitudinal variations (80 m -170 m) and showed no significant relationship between density and altitudes in contrast to CDC-light traps which showed a significant negative impact on capturing efficacy with increase in altitudes (P<0.001).

Conclusions: The study suggests that BG-Sentinel traps can be used effectively and efficiently to collect more *Ar. subalbatus* mosquitoes during night time in comparison to CDC-light trap under complex climatic conditions of forest and variable altitudes.

1. Introduction

Area surveillance is essential to determine the occurrence of the targeted insect pests and to understand the population dynamics in time and space which plays a key role in decision making for vector control operations[1]. The highly sensitive and accurate surveillance method provides considerable estimates of the population density whereas

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incorrect interpretations of population dynamics may lead to failure of the control program[2,3].

The surveillance of adult mosquito has always been tedious, costly and less effective due to unavailability of highly attractive adult traps. The BG-Sentinel and CDC-light traps are considered as standard for mosquito surveillance that utilizes light, odour and colours[4-6]. Both traps have been used extensively in various conditions and found effective in collecting many different kinds of mosquito species[7-9]. Although, the light traps are only used for nocturnal mosquitoes including *Anopheles* and *Culex* whereas chemical lure based BG-Sentinel traps are preferred to collect day active *Aedes* mosquitoes[10-12]. These traps have been mostly used in urban, suburban and rural areas where *Anopheles*, *Culex* and *Aedes* are prevalent[13,14].

Armigeres (Ar.) subalbatus (Coquillett) is one of the important mosquito species transmitting filarial worm, *Brugia pahangi* and other related species in Asian countries[15,16]. It prefers human blood over animals and shows bimodal peaks of serious biting problem caused by biting rhythms[17-19]. *Ar. subalbatus* is mainly a forest mosquito and becomes abundant during the rainy season[20-22]. It has been well established in a variety of urban habitats[23]. However, there is no effective surveillance method specifically known to assess realistic population density of adult *Ar. subalbatus* mosquito under variable habitats.

Until now, the surveillance of adult *Ar. subalbatus* mosquitoes depends on inefficient and non-realistic approaches such as outdoor light traps and indoor resting aspirator collections which provide underestimated counts. In the present study, we seek whether BGS traps developed for diurnal *Aedes* mosquitoes can provide a better tool to collect large populations of *Ar. subalbatus* mosquitoes as opposed to CDC-light traps and at the same time are able to nullify the minor altitudinal variations in complex forest habitats. To test this hypothesis, comparative studies of BG-Sentinel and CDC-light traps for *Ar. subalbatus* were conducted in protected forest areas of West Bengal, India. The outcomes of the study provide significant data on the implications of BG-Sentinel traps with a chemical lure for the surveillance of forest mosquitoes.

2. Materials and methods

2.1. Study area

The present study was conducted in three protected forest areas located in lower northeastern Himalayan regions of West Bengal, India: Neora Valley National Park (NP), Gorumara National Park (Gorumara NP) and Mahananda Wildlife Sanctuary (WLS) (Supplementary Figure 1, Supplementary Table 1). Neora Valley NP is distributed over an area of 88 km in Kalimpong district, West Bengal is known for the elegant red panda found in the pristine undisturbed natural habitat with rugged inaccessible hilly terrain and rich diverse flora and fauna. Gorumara NP is located in the eastern Himalayas' sub-mountain Terai belt in Jalpaiguri district and designated as natural breeding habitats of Indian rhinoceros encompassing about 80 km^2 area. Whereas, Mahananda WLS is located on the foothills of the Himalayas, between the Teesta and Mahananda rivers in Darjeeling district covering 159 km^2 of the reserve forest designated to protect the Indian bison and Royal Bengal tiger. All the reserved forest areas provide scenic attractions and recreation facilities to the tourists from within and outside India. Thus, these locations were of prime importance regarding interactions of mosquito vectors, local residents and tourists which may act as reference sites for future studies on vector-borne disease surveillance.

For the surveillance of mosquitoes, five locations including camps and human dwellings were selected in each forest area with the permission of the forest department, West Bengal and subsequently from the homeowner and the property owners to deploy the traps. These houses, offices and towers were surrounded with trees and shrubs that can provide suitable resting habitat to adult mosquitoes near the blood-feeding host. At many places, domestic animals such as cattle or cow sheds were also present near human premises that help in the attraction of more host-seeking mosquitoes. There were water cisterns, small rivers, riverine, pools, puddles and road construction site present that may support larval breeding of mosquitoes. Our preliminary studies have shown the presence of *Aedes (Ae.) albopictus*, *Anopheles* spp. and *Culex quinquefasciatus* larvae in construction sites and discarded containers in human premises. Each location was marked with GPS coordinates and altitudes (Supplementary Table 1). These coordinates were subjected to Google Earth Pro software 2018 ver. 7.3.2 (California, U.S.A). for the presentation of sampling locations in the map (Supplementary Figure 1). The sampling sites in Neora Valley NP were denser human inhabitation at Fari Basti and Mondal gram locations in comparison to other sites, whereas Gorumara NP and Mahananda WLS sites included mostly watch towers where two to three forest guards were present at all the time except for residential locations such as Lataguri and Range Office of Gorumara NP, respectively (Supplementary Table 1).

2.2. Mosquito traps and sampling

For mosquito sampling, five CDC-miniature light traps (CDC-LT) (John W. Hock Company, Gainesville, FL, USA) and five BG-Sentinel-Ver. II (BGS) traps (Biogents AG Inc., Regensburg, Germany) were deployed during the last week of May and the first week of June 2018. The traps were deployed for 12 h period between ~ 6:00 PM and ~ 6:00 AM for night collections to compare the efficacy of both traps. All the traps were labelled with locations name and trap type (BGS/LT) *i.e.* RO-BGS – Range Office, BG-Sentinel, RO-LT – Range Office, CDC-LT trap. This labelling system was followed across all the sampling locations. At each sampling location, one BGS and one CDC-LT were installed in a pair at the marked trap site. All the traps were repeatedly placed for three consecutive nights with rotation between BGS and CDC-LT sites as well as different locations following the Latin square design to avoid site based biases in the data. Sampling sites were selected based on information obtained from the villagers and homeowners regarding mosquito prevalence. Efforts were made to cover the entire altitudinal range of the sampling area by considering five trap locations. The locations included human residence, guest houses and forest guard watch towers in the forest where human hosts were present during sampling. CDC-LT traps were hanged on tree branches or on porch frames of the house shed 150 – 210 cm above ground level whereas BGS traps were placed on the floor under the tree or large bushes as described by the manufacturer. Both traps were at least 25 m distant from each other. Efforts were made to avoid the direct bright light exposure from bulbs or fluorescent tubes. For uninterrupted operation, both CDC-LT and BGS traps were powered with 6 V and 12 V DC rechargeable batteries, respectively. BGS traps were equipped with BG-lure and a new lure cartridge was used for each location whereas no lure was supplemented in CDC-LT. Mosquitoes caught in the traps were mouth aspirated and gently transferred into plastic bottles labelled with location, date and trap type. Total numbers of mosquitoes including dead from each trap were recorded and identified to species level using the taxonomic keys of Barraud[24]. The collected mosquitoes were transported to the laboratory at Kolkata for processing and preservation in the repository collection for further experimentations. A 10% sugar solution was provided on cotton wicks to adults for survival.

2.3. Weather data

The data on the minimum and maximum temperature and rainfall were collected for Neora Valley NP at Samsing Tea Gardens, Gorumara NP at Lataguri and Mahananda Wildlife Sanctuary at Siliguri using online weather website for the sampling day (https://weather.com/en-IN/weather/monthly). Neora Valley NP area showed 11.0 ℃ and 21.3 ℃ as a minimum and maximum temperatures, respectively and received 0.21 cm rainfall. Gorumara NP showed 11.3 ℃ and 22.67 ℃ as a minimum and maximum temperatures, respectively with a 0.45 cm rainfall. The minimum and maximum temperatures of Mahananda WLS were 25.8 ℃ and 34.0 ℃, respectively and had 0.12 cm rainfall. These temperatures were the average for minimum and maximum during sampling days and rainfall represents cumulative precipitation. During the sampling nights, the weather was cloudy and received slight rainfall on seven nights out of nine. The moon phase was a full moon at first night of the sampling with 100% illumination that reduced to 97% at waning gibbous moon during the 3rd night at Neora Valley NP (www.moongiant.com). At Gorumara NP, 1st-night moon illumination was 92% with waning gibbous moon phase that reduced to 79% at 3rd night of sampling with a similar moon phase. The moon illumination was 71% with waning gibbous at 1st night of sampling that was reduced to 53% illumination at last quarter waning gibbous moon at the 3rd night of sampling. However, the background light intensity was not measured for individual trap locations as dark areas were selected for trap placement.

2.4. Statistical analysis

Mosquitoes collected in CDC-LT and BGS traps from different locations on various days were converted to density (mosquito/trap/night). For the respective trap, density data on consecutive days were considered as a replicate and pooled for the statistical analysis. Before analysis, the data were subjected to normality distribution using curve. Multiple factor analysis of variance (ANOVA) for the density of mosquitoes was conducted to assess the significant differences among the traps, locations and days. In the analysis, interactions between locations and traps were assessed as main variants and days were the covariates ($P<0.05$). Significant differences between variables were estimated with a multiple range test method using Fisher's Least Significant Difference at $P<0.05$. The impact of altitudes on the efficacy of BGS and CDC-LT traps was assessed by comparison of slopes of regression lines between mosquito density and altitudes using multiple regression analysis ($P<0.05$). For the regression analysis, mosquito density data from all the sampling locations were pooled and transformed using log10(y+1) transformations. The regression equation for the density with altitudes and R-squared values for all the locations were estimated. In the analysis, intercepts of the regression line were assumed equal for the comparison of the regression slopes for statistical differences. For the statistical analysis, Statgraphic Plus Version 5 (Statistical Graphic Corp., Virginia, USA) software was used and the graphs were prepared in Microsoft Office version 2013. The data were expressed as mean ± SE unless otherwise mentioned.

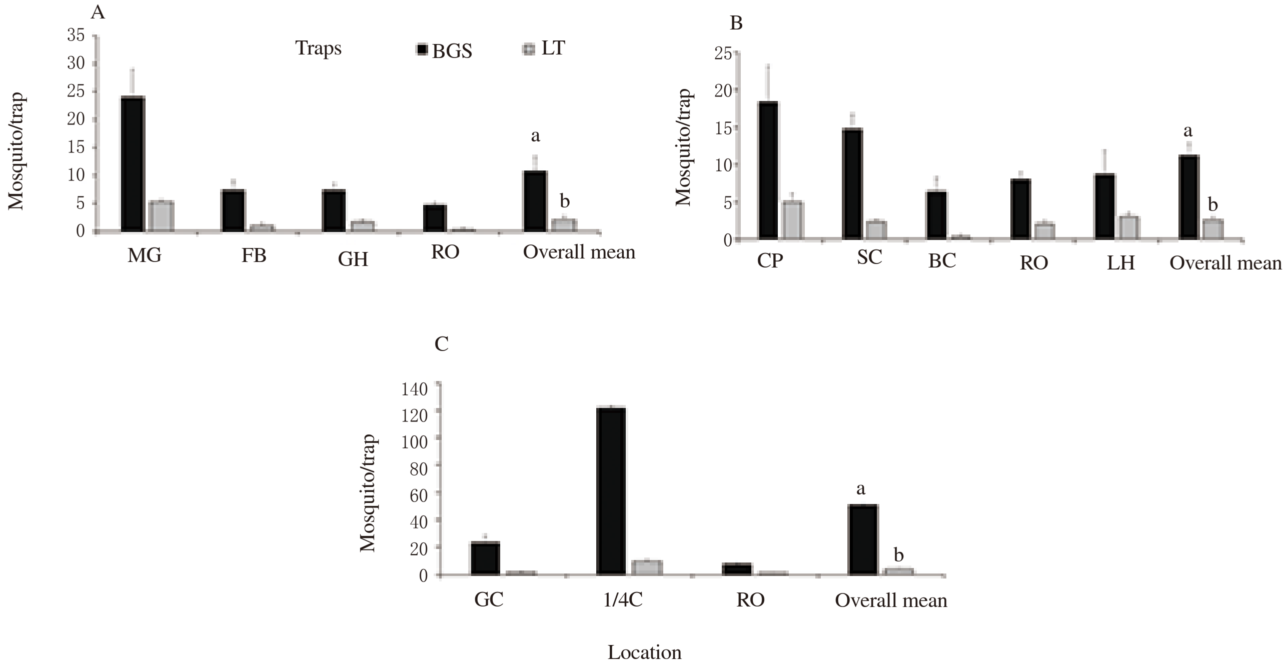

Figure 1. Comparative efficacy of BG-Sentinel trap (BGS) and CDC-light trap (LT) for adult *Armigeres subalbatus* mosquito collection in three forest reserves in West Bengal, India. (A) Neora Valley National Park: MG-Mandal Gram, FB–Fari Basti, GH–Forest Guest House, RO–Range office, (B) Gorumara National Park: CP–Check post, SC–Sambhar Camp, BC–Bamni camp, RO–Range Office, LH–Lataguri, and (C) Mahananda Wildlife sanctuary: GC-Colaghat camp, 1/4C–camp, RO–Range Office. Both Traps caught most mosquitoes in MG than they did in other locations. Bars denoted with different letter differ significantly (P<0.05) and error bar represents standard error (SE).

3. Results

3.1. Mosquito collection and species prevalence

A total of 905 adult mosquitos were collected from Neora Valley NP, Gorumara NP and Mahananda WLS during the study in both BGS and CDC-LT which contains 857 (94.69%) *Ar. subalbatus* as a major proportion in comparison to both 12 (1.33%) *Ae. albopictus* and 36 (3.98%) *Culex quinquefasciatus*. BGS traps collected all the three species whereas CDC-LT did not receive any *Ae. albopictus* from any location.

3.2. The density of adult *Ar. subalbatus* at different forest area reserves

Ar. subalbatus mosquitoes were consistently collected in both CDC-LT and BGS from all the sampling locations. In Neora Valley National Park, the mosquito density ranged between [(4.67±0.67) and (24.00±4.93) mosquito/trap/night] in BGS traps and [(0.33±0.33) and (5.33±0.33) mosquito/trap/night] in CDC-LT traps. The average mosquitoes density in BGS traps were significantly higher [(10.83±2.59) mosquito/trap/night] in comparison to CDC-LT traps [(2.08±0.61) mosquito/trap/night] (Multifactor ANOVA, DF = 1, F value = 43.77, P<0.001; Figure 1A, Table 1). Among the various locations, mosquito density was significantly higher at Mondal Gram in Neora Valley. In Gorumara NP, the density of *Ar. subalbatus* ranged between [(6.33±1.86) and (18.33±4.84) mosquitoes/trap/night] in BGS traps and [(0.33±0.33) and (5.00±1.54) mosquitoes/trap/night] in CDC-LT traps. The mean density of mosquito was significantly higher (11.20±1.64) in BGS trap than the CDC-LT traps (2.53±0.48) (Multifactor ANOVA, DF = 1, F value = 43.88, P<0.001; Figure 1B, Table 2). In Gorumara NP, the Check post area showed significant higher density in both BGS and CDC-LT traps in comparison to the other three locations except for Sambhar camp (Figure 1B). Mahananda Wildlife Sanctuary is located in the lower altitudinal region than Neora Valley with hilly and undulating terrains, but higher than Gorumara NP (Supplementary Figure 1, Supplementary Table 1). In BGS traps, the mean density of mosquito [(50.89±18.99) mosquito/trap/night; range: (7.67±0.88) and (121.67±22.37) mosquitoes/trap/night] was significantly higher than the mean mosquito density [(4.22±1.69) mosquito/trap/night; range: (1.00±0.58) and (10.00±2.89) mosquitoes/trap/night] in CDC-LT traps at Mahananda WLS (Multifactor ANOVA, DF = 1, F value = 46.25, P<0.001; Figure 1C, Table 3).

Table 1. Multifactor analysis of variance (ANOVA) among the density of *Armigeres subalbatus* mosquito, locations and sampling day to estimate the efficacy of BG-Sentinel trap in lower Neora Valley National Park, West Bengal, India.

Source	Sum of squares	*DF*	Mean square	*F* value	*P* value
Day*	22.563	1	22.563 0	2.15	0.163 3
A:Locations#	552.792	3	184.264 0	17.56	0.000 1
B:Trap#	459.375	1	459.375 0	43.77	0.000 1
AB$	199.792	3	66.597 2	6.35	0.005 4
TOTAL (corrected)	1 391.960	23			

Symbols: * - Covarieties, # - Main effects, $ - Interactions

Table 2. Multifactor analysis of variance (ANOVA) among the density of *Armigeres subalbatus* mosquito, locations and sampling day to estimate the efficacy of BG-Sentinel trap in Gorumara National Park, West Bengal, India.

Source	Sum of squares	*DF*	Mean square	*F* value	*P* value
Day*	22.050	1	22.050	1.72	0.205 7
A:Locations#	256.467	4	64.117	4.99	0.006 4
B:Trap#	563.333	1	563.333	43.88	0.000 1
AB$	87.667	4	21.917	1.71	0.190 0
TOTAL (corrected)	1 173.470	29	-	-	-

Symbols: * - Covarieties, # - Main effects, $ - Interactions

3.3. Impact of time and locations on the efficacy of traps

There were significant interactions observed between the locations and traps at Neora Valley NP, however, the time was not the significant factor to impact efficacy of the traps (Table 1). At Gorumara NP, the interaction was not significant between locations and trap efficacy, and sampling time was not a significant factor (Table 2). However, the efficacy of traps was significantly interacted with sampling locations at Mahananda WLS (P<0.001) (Table 3).

Table 3. Multifactor analysis of variance (ANOVA) among the density of *Armigeres subalbatus* mosquito, locations and sampling day to estimate the efficacy of BG-Sentinel trap in Mahananda Wildlife Sanctuary, West Bengal, India.

Source	Sum of squares	*DF*	Mean square	*F* value	*P* value
Day*	784.083	1	784.083	3.70	0.080 6
A:Locations#	13 386.800	2	6 693.390	31.59	0.000 1
B:Trap#	9 800.000	1	9 800.000	46.25	0.000 1
AB$	9 675.000	2	4 837.500	22.83	0.000 1
TOTAL (corrected)	35 976.400	17	-	-	-

Symbols: * - Covarieties, # - Main effects, $ - Interactions

3.4. Impact of altitudes on the efficacy of the traps

In the present study, the sampling locations were distributed between 82 m to 920 m altitudes; Neora Valley NP ranged from 247 m to 920 m, Gorumara NP ranged from 82 m to 118 m, and Mahananda WLS ranged from 138 m to 169 m (Supplementary Figure 1, Supplementary Table 1). The impact of altitudes on the efficacy of BGS and CDC-LT traps indicated that the density in BGS trap was not reduced with the increase in altitudes, whereas CDC-LT trap showed a significant reduction in density of *Ar. subalbatus* mosquitoes with an increase of altitude (Figure 2, Table 4).

Table 4. Impact of altitudes (X-axis) on the efficacy of BGS and CDC-LT traps (Y-axis) for *Armigeres subalbatus* mosquito using multiple regression analysis for the comparison of regression line slopes in forest areas, West Bengal, India.

(A) Multiple regression analysis.

Source	Sum of squares	*DF*	Mean square	*F* value	*P* value
Altitude	0.035 936 4	1	0.035 936 4	0.21	0.651 5
Slopes	3.736 770 0	1	3.736 770 0	21.40	0.000 1
Model	3.772 710 0	2	-	-	-

Regression line slopes in forest areas, West Bengal, India.

(B) Further ANOVA for variables in the order fitted.

Parameter	Estimate	Standard error	*T* value	*P* value
Constant	0.823 168 000	0.072 279 400	11.388 7	0.000 1
Altitude* BGS trap	0.000 446 882	0.000 200 414	2.229 8	0.029 0
Altitude* LT Trap	-0.001 044 020	0.000 225 709	-4.625 5	0.000 1

Symbol: * - represents the relationship between the corresponding factors.

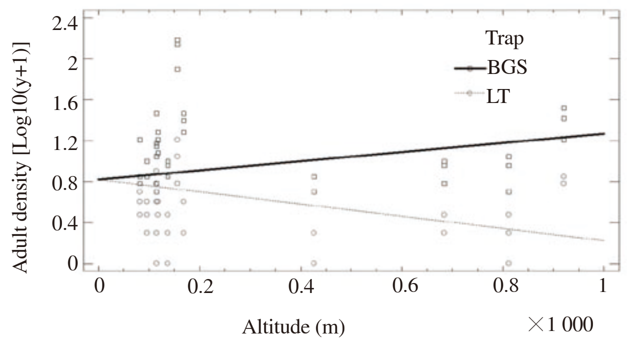

Figure 2. Effect of geographical altitudes (X-axis) on the efficacy of BG-Sentinel and CDC-light traps as the adult density of *Armigeres subalbatus* (Y-axis) using multiple regression for the comparison of slope in forest areas of West Bengal, India.

4. Discussion

The realistic and accurate data collection on mosquito population density is one of the most important parameters for the management of vector-borne transmitted diseases. Effectiveness and sensitivity of a surveillance method are directly related to the accuracy in the analysis of the population dynamics and success rate of the vector control program[3]. In the present study, we investigated chemical-lure based BG-Sentinel traps, developed for diurnally active mosquitoes *i.e. Ae. aegypti* and *Ae. albopictus*, to find an effective and sensitive surveillance method for nocturnal adult *Ar. subalbatus* populations predominantly found under humid forest climates in comparison to the standard CDC-miniature light traps. The CDC-light trap has been used for night mosquito surveillance for several decades[4]. We found that the performance of the BGS trap was much greater than CDC-LT trap in terms of night collection of *Ar. subalbatus* mosquitoes at various altitudes in forest conditions. It shows that without having an effective trap, the observed density may be underestimated.

The forest habitat provides complex situations because of interactions of many variables such as humidity, sunlight penetration, shade and coverage with trees and bushes (http://www.fao.org/3/XII/1018-B2.htm). Such altitudinal variations in undulating forest areas affect many environmental factors that might affect the distribution of the species and density[25]. We have noted variations in temperature and altitude among Neora Valley NP located at high altitudes (427 m – 920 m) which experiences lower temperature regimes (11 ℃ – 21.3 ℃) than Mahananda WLS located at lower altitudes (138 m – 169 m) with higher temperature (25.8 ℃ – 34.0 ℃). These variations have impacted the mosquito species prevalence and density distribution in these forest areas as we have found that the mean density of *Ar. subalbatus* was higher in Mahananda WLS in comparison to Neora Valley NP.

An efficient trap can be helpful to obtain the accurate mosquito density in an area[26]. It has been found that the divergence in the behavior of adult mosquito affects the efficacy of the traps[26]. Several workers have evaluated various traps *i.e.* mosquito magnate, light trap, BGS traps, with modified designs and combinations of lures, which showed enhancement in trap efficacy to capture the high density of mosquitoes with multiple cues[27-29]. BG-Sentinel traps have been mostly used against the container-breeding Aedes mosquitoes[28-31]. The present study explors the field of using BGS traps to assess the density of *Armigeres* mosquitoes in the forest condition. *Ar. subalbatus* is highly prevalent in the forest habitats and have been established in the semi-urban and urban areas and has shown predominant crepuscular activity[23]. It has been known that the light traps performed better to collect mosquitoes under night condition[5]. However, we have shown that BGS traps perform much better in collecting adult *Ar. subalbatus* mosquitoes in higher density from all locations during the night in comparison to light traps in forest areas. This suggests that BGS traps can be used to collect mosquitoes efficiently in the night time and in complex habitats such as a forest. The study also suggests evaluating BGS traps for other forest mosquitoes to develop an efficient surveillance tool.

The moonlight has shown a negative impact on light trap efficacy against the night active mosquitoes[26]. In our study, the efficacy of CDC-LT might be impacted with complex light conditions existed during the sampling as the moon was present with 53%–100% illumination. However, it is tough to assess the impact accurately as weather was cloudy and traps were placed under the tree and shed to avoid the exposure of light. Moreover, this mosquito was more active during dusk and an early night, so, moonlight impact might be minor.

Uniform distribution of mosquitoes is almost impossible to achieve in forest conditions due to a patchy distribution of human residences, undulating areas, variable tree coverage and scattering larval habitats[32]. In line with the observations of Lord[32], we have also found that Mondal Gram had a higher density than the other three locations in the Neora Valley NP; Check post area was denser than all other four locations in Gorumara NP, and ¼ Campsite contained the highest mosquito density in Mahananda WLS. All these locations are occupied by human premises which may provide additional larval habitat, however, we did not find larval habitats in close range. The rest of the study locations were almost similar in density without any statistical difference indicating the prevalence of *Ar. subalbatus* predominantly in the forest in the months of May and June. Based on our observation we suggest that BGS traps should be evaluated in different habitats at different seasons to identify the impact of habitats and seasonal variations on the prevalence of *Ar. subalbatus*.

Despite variations in the density of *Ar. subalbatus* at different locations, BGS trap performed better in handling the impact of altitudes on the mosquito distribution in comparison to CDC-LT. It is possible that the light cues to host-seeking mosquitoes are weaker than the chemical lure which provides host resemblance and able to attract more mosquitoes that nullifies the variations. The regression analysis between the density of mosquito and altitudes confirms that the efficacy of BGS traps was not affected at a lower altitudinal range located in Gorumara NP and Mahananda WLS in comparison to the Neora Valley NP which is located at higher altitudes (data not shown). In contrast, CDC-LT showed significant impacts of altitudinal variations on the efficacy at all three locations irrespective to the sampling areas suggesting that BGS trap is a better alternative option to traditional CDC-LT for the surveillance of nocturnal mosquitoes in forest areas. Further studies are needed to explore the efficacy of BGS traps with different lure compositions for other vector mosquitoes in forest areas.

Apart from the high efficacy of BG-Sentinel traps, we have noted high condensation of moisture in the cartridge that deformed the active attractant lure material and free-flow granular lure crystals were turned into thick and jelly-like material. There is a need to improve the lure cartridge to make it long-lasting under high humidity conditions.

The present study provides significant data that can help in search of an effective adult mosquito trap alternative to light traps which is less efficient and sensitive to climatic variables. The chemical lure based BGS trap performed superiorly over the CDC-LT to collect adult *Ar. subalbatus* mosquitoes in the forest areas of Neora Valley NP, Gorumara NP and Mahananda WLS located at West Bengal, India during night collections. BGS traps also performed better under optimum altitudinal variations where the efficacy of CDC-LT was significantly affected. BGS traps can be used when a large number of mosquitoes are required to do molecular analysis or parasitic detection present in the forest conditions. Further improvement in lure cartridge is suggested to use in high humid conditions.

Conflict of interest statement

The authors declare that they have no competing and conflict of interest.

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