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A Comparative Evaluation of Cattle-Baited and Alternative Trap Methods for Sandfly Surveillance in a Leishmaniasis-Endemic Setting in Matale District, Sri Lanka

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Abstract:

- ***Background:***

Leishmaniasis, transmitted by female *Phlebotomus* sandflies, poses a growing threat in South Asia, including Sri Lanka, where cutaneous forms predominate and *Ph. argentipes* is the primary vector. Despite rising incidence in hotspots like Matale District, gaps persist in vector ecology, density, seasonality, behavior, and surveillance efficiency hindering targeted control. Comparative trap evaluations are essential to optimize monitoring amid behavioral heterogeneity.

- ***Methods:***

From August 2024 to December 2025, a longitudinal survey was conducted monthly in leishmaniasis-endemic peridomestic sites in Kandalama area, Matale District, Sri Lanka. Seven methods were deployed: cattle-baited traps (CBTC), light traps (LT), sticky traps (ST), indoor/outdoor hand collections, indoor double-net traps, and outdoor double-net traps. Female sandflies were morphologically identified to genus (*Phlebotomus* vs. *Sergentomyia*). Negative binomial GLMMs (glmmTMB) assessed trap efficiency yielding incidence rate ratios (IRRs).

- ***Results:***

Of 3,686 females collected, *Phlebotomus* spp. comprised 79.5% (n=2,929), peaking in CBTC (1,366; 46.6%). *Sergentomyia* spp. (20.5%; n=757) favored indoor hand collections (213; 28.1%). CBTC excelled (mean 952/effort; ref.), outperforming others (e.g., ST IRR=0.0016 [95%CI 0.0006–0.004]; LT mean=265 [121–583]; all p<0.001). *Sergentomyia* showed lower abundance (IRR=0.26–0.36; p<0.002). *Phlebotomus* exhibited pronounced late-year peaks.

- ***Conclusions:***

CBTC is optimal for *Ph. argentipes*-focused surveillance in livestock settings, capturing genus biases and seasonality critical for leishmaniasis early warning. Integrating such data refines risk models, informing interventions in tropical hotspots.

Keywords: *Phlebotomus Spp., Sandfly Surveillance, Cattle-Baited Trap, Trap Efficiency.*

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I. INTRODUCTION

Leishmaniasis, a vector-borne disease caused by protozoan parasites of the genus Leishmania, imposes a substantial global public health burden through its cutaneous, mucocutaneous, and visceral forms(Arenas et al., 2017; Khosravani et al., 2016; Rahim & Karim, 2024).

Female phlebotomine sand flies (Diptera: Psychodidae), primarily of the genus Phlebotomus, serve as the principal vectors, transmitting pathogens during blood meals(El Ghrifi et al., 2024; Wijerathna et al., 2021, 2022). In South Asia, including Sri Lanka, Ph.argentipes predominates as the key vector for L. donovani, the causative agent of cutaneous leishmaniasisthe dominant clinical form in the region(Kariyawasam et al., 2017; H. Siriwardana et al., 2010; H. V. Y. D. Siriwardana et al., 2007). While historically considered an imported disease in Sri Lanka prior to the 1990s, leishmaniasis has since become established, with incidence increasing substantially over the past two decades. The Ministry of Health, Sri Lanka has declared it a notifiable disease in 2008(Hewawasam et al., 2020) and however, fundamental understanding of vector biology remains limited compared to epidemiological knowledge (Kumari et al., 2026).

Effective management is hindered by this discrepancy because focused interventions are undermined by gaps in knowledge of sand fly ecology, which includes density, regional distribution, seasonal abundance, resting locations, host-seeking behavior, and population genetics(Amarasinghe & Wickramasinghe, 2020; Wedage et al., 2025). Early insecticide tolerance and genetic heterogeneity in Ph. argentipes have been reported in a small number of previous investigations conducted in Sri Lanka, suggesting regional adaptations that may influence transmission dynamics(Pathirage et al., 2020, 2021). Comprehensive field data that include these characteristics is still hard to come by, especially in developing areas. Matale District in Central Sri Lanka is a prime example of this deficiency(Amarasinghe & Wickramasinghe, 2020; Gunathilaka et al., 2025); according to recent surveillance, it is a hotspot for cutaneous leishmaniasis, which is associated with peri-urban populations, small-scale animal farming, and humid agro-ecological ecotones that are favorable to synanthropic sand flies(Galgamuwa et al., 2018). Although morphologically distinct Ph. argentipes populations occur in transmission zones, inadequate data on vector density, seasonality, and behavior limit evidence-based management strategies (Wedage et al., 2025).

Effective sand fly surveillance demands diverse trapping methods to capture behavioral heterogeneity, as no single technique fully represents population dynamics or host preferences. Common approaches include cattle-baited traps (CBTC), light traps (LT), sticky traps (ST), human landing collections (HLC), and hand captures (indoor/outdoor)(Cameron et al., 1991). Sri Lankan studies affirm their utility across rural settings, revealing climate-driven seasonal peaks in warm, humid periods(Herath et al., 2021; Senanayake et al., 2015). Nonetheless, comparative evaluations under local conditions quantifying trap efficiency, species composition, and links to disease incidence are rare (Senanayake et al., 2015).

Against this backdrop, the present study conducts a comparative evaluation of cattle-baited traps (CBTC) against alternatives including light traps (LT), sticky traps (ST), human landing collections (HLC), indoor/outdoor hand capture for sand fly surveillance in leishmaniasis-endemic Matale District, Sri Lanka. The core objectives of the study are: (1) quantify relative abundance and species composition (Phlebotomus vs. Sergentomyia) across trap types; and (2) delineate monthly/seasonal abundance patterns relative to standardized effort. By elucidating trap-specific efficiencies and ecological insights, this entomological investigation fills pivotal gaps, enabling tailored vector control and enhancing leishmaniasis mitigation in Sri Lanka and analogous tropical settings.

II. MATERIALS AND METHODS

➢ *Study Area*

An entomological survey was conducted in the Kandalama area within the Dambulla Medical Officer of Health (MOH) division in Matale District, Sri Lanka, where a high clustering of leishmaniasis cases had been reported. The study covered five Grama Niladhari (GN) divisions: Kandalama 25 Colony, Kandalama 29 Colony, Yakuragala North, Yakuragala South, and Wilhatha. These areas were selected based on the spatial distribution of confirmed human cases.

➢ *Study Design and Sampling Strategy*

A longitudinal survey was carried out from August 2024 to December 2025. Sampling was conducted over four consecutive days each month in peridomestic environments located in close proximity to reported patient households. Site selection was purposive, targeting high-risk areas with recent case reports.

➢ *Sand Fly Collection Methods*

Multiple techniques were employed to maximize sand fly collection and capture different behavioral patterns:

- Light traps were deployed outdoors in peridomestic settings.
- Cattle-baited trap was used to attract zoophilic sand flies.
- Sticky traps (handmade using white papers and oil) were placed in outdoor locations to passively collect resting and flying sand flies.
- Procopack aspirators were used for indoor and outdoor collections to capture resting sand flies.
- Full-night human landing collections using double-net trap was conducted from 18:00 to 06:00 hours to determine biting activity and peak abundance hours.

➢ *Specimen Handling and Processing*

Collected sand flies were transported as dry specimens to the Regional Malaria Laboratory, Matale, for further processing. In the laboratory, specimens were first sorted by sex based on morphological characteristics.

Subsequently, sand flies were separated into genera, primarily Phlebotomus and Sergentomyia, using standard taxonomic features. Genus-level identification was performed using established morphological keys(Wijerathna & Gunathilaka, 2020).

- *Slide Preparation and Identification*

Selected specimens were processed following standard slide-mounting procedures. Specimens were passed through a graded ethanol series for dehydration, cleared using lactophenol and permanently mounted on microscope slides using Hoyer's mounting medium.

Morphological identification was carried out under a compound microscope, focusing on key diagnostic features such as antennal segments, cibarium, pharynx, and genitalia. Only the morphological identification was used in this study.

- *Statistical Analysis*

Data were imported and prepared in R (version 4.5.3) using the tidyverse, lubridate, and glmmTMB packages. Year, species, and trap type were treated as categorical variables, and the month factor was transformed to sinusoidal terms to model seasonality. Sampling effort was log-transformed as an offset. Monthly sandfly abundance was aggregated by date, with total counts and effort summed to compute rates per unit effort. Lagged sandfly rates (0–6 months) were generated using dplyr::lag(), and descriptive statistics were summarized.

Trap efficiency was evaluated using a negative binomial generalized linear mixed model (GLMM), with sandfly count as the response, log (effort) as offset, and fixed effects for species, year, and trap type. Random intercepts for year and month accounted for temporal clustering and overdispersion. Incidence rate ratios (IRRs) were obtained by exponentiating coefficients and pairwise trap type comparisons was done by estimated marginal means on the log scale, back-transformed with Tukey-adjusted p-values. Temporal sandfly population dynamics were explored by extending the primary model to test the interaction of species and month. The fixed effects summarized as IRRs (95% CI) and visualized as marginal means with confidence intervals.

III. RESULTS

A total of 3,686 female sandflies were collected across seven trap types, comprising 2,929 Phlebotomus spp. (79.5%) and 757 Sergentomyia spp. (20.5%) (Table 1). The cattle baited trap yielded the highest number of Phlebotomus spp. (1,366 individuals; 46.6% of total Phlebotomus), with no Sergentomyia spp. detected. Double net traps performed strongly overall, capturing 286 Phlebotomus spp. (9.8%) and 84 Sergentomyia spp. (11.1%) indoors, and 976 Phlebotomus spp. (33.3%) and 194 Sergentomyia spp. (25.6%) outdoors. In contrast, hand collection methods favored Sergentomyia spp., particularly indoors (213 individuals; 28.1% of total Sergentomyia) despite low Phlebotomus yields (29 indoors, 76 outdoors). Light traps (127 Phlebotomus spp., 196 Sergentomyia spp.) and sticky traps (69 Phlebotomus spp., 6 Sergentomyia spp.) contributed modestly to collections.

Phlebotomus spp. dominated in animal-baited and net-based traps, while Sergentomyia spp. were more prevalent in hand collections and light traps. Trap-specific proportions highlight differential efficiencies: cattle baited traps exclusively captured Phlebotomus spp., whereas hand collection (indoor) yielded 88.0% Sergentomyia spp.

The GLMM results confirmed that the trap type strongly influenced capture rates, with the CBTC serving as the reference and yielding the highest mean counts (952 per unit effort; 95% CI: 410–2209). All other trap types showed significant reductions relative to CBTC ($p<0.001$) (Figure 1 and 2), including double net traps yielded intermediate performance (indoor: IRR=0.06, 95% CI 0.02–0.17; outdoor: IRR=0.16, 95% CI 0.06–0.42; both $p<0.001$), while light traps were moderately effective (IRR=0.29, 95% CI 0.09–0.81; $p<0.05$). Hand collections performed poorly (indoor: IRR=0.06, 95% CI 0.02–0.20; outdoor: IRR=0.04, 95% CI 0.01–0.13; both $p<0.001$), and sticky traps were least efficient (IRR<0.001, 95% CI 0.00–0.00; $p<0.001$), with predicted means 1,000-fold below CBTC (Table 2).

Sergentomyia spp. exhibited 64% lower abundance than Phlebotomus spp. (IRR=0.36, 95% CI 0.18–0.69; $p<0.001$), underscoring genus-specific trap biases. Model diagnostics indicated good fit, with dispersion parameter ≈1.03 ($p>0.64$) and minimal zero-inflation (Table 2). The total collection rates of sandflies showed peak collection during later months of the year, with Phlebotomus sp. showing greater amplitude (Figure 3).

IV. DISCUSSION

The superior performance of the cattle baited trap collection (CBTC) relative to other methods was observed across both Phlebotomus sp. and Sergentomyia sp., highlighting its efficacy in sampling zoophilic sandfly populations. This finding aligns with studies on Anopheles vectors, where cow-baited tents captured 10- to 20-fold more individuals than human landing collections or light traps, attributed to the strong attraction of host odors such as ammonia and lactic acid emitted by cattle(Marasri et al., 2017; Senanayake et al., 2015). Such animal-baited approaches are thus recommended for surveillance in rural, livestock-associated settings, as they minimize human vector exposure while maximizing yield (Laurent et al., 2016)

Lower capture efficiencies in alternative traps, including sticky traps and light traps, were evident particularly for Phlebotomus sp., though light traps showed relative utility for Sergentomyia sp. These patterns reflect species-specific behavioral preferences, with Phlebotomus sp. exhibiting stronger zoophily and endophagy (Bursali& Touray, 2024)compared to the more exophilic Sergentomyia sp., consistent with ecological reviews of sandfly foraging strategies. The poor performance of sticky traps across taxa underscores their limitations in low-density environments. To increase the catch, the modified sticky trap can be utilized (Elnaiem et al., 2020).

Temporal dominance of Phlebotomus sp. and its pronounced seasonality were linked to warmer months, suggesting temperature-driven reproductive and dispersal dynamics(Karmaoui, 2020; Karmaoui et al., 2022). This synchrony parallels mosquito vector studies, where abundance peaks correlate with optimal thermal thresholds for gonotrophic cycles(Mala et al., 2014). Non-linear monthly trends further indicate multifaceted environmental regulation, potentially involving humidity or photoperiod, warranting integrated climatic modelling in future surveillance.

These results underscore trap selection and temporal monitoring as critical for leishmaniasis early warning systems, with CBTC optimizing detection of high-risk Phlebotomus sp. populations. Integration with case surveillance enhances predictive accuracy, though broader covariates like vector competence and host immunity merit exploration to refine risk models. Future studies should validate these patterns across diverse eco-epidemiological zones.

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- *Declaration of Conflict of Interest*

The authors declare no competing interests.

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TABLES AND FIGURES

Table 1 Number of Phlebotomus spp. and Sergentomyia spp. Sand Flies Collected by Trap Type in the Study Area.

Trap type	**Phlebotomus spp**	**Sergentomyiaspp**
Cattle baited trap	1366	0
Double net trap (indoor)	286	84
Double net trap (outdoor)	976	194
Hand collection (indoor)	29	213
Hand collection (outdoor)	76	64
Light trap	127	196
Sticky trap	69	6
Total	2929	757

Table 2 Results From a Generalized Linear Mixed Model (GLMM) Examining Factors Associated with Sandflyabundance. Coefficients Represent log-Transformed Estimates, with IRR (Incidence Rate Ratios) and 95% Confidence Intervals (CI) Derived from Exponentiation. Significant p Values are Marked Relative to Reference Categories (CBTC for Collection Methods; Phlebotomus spp. for Sand fly Species)

Variable	**Estimate**	**Std_Error**	**IRR**	**CI_lower**	**CI_upper**	**P value**
(Intercept)	2.86	0.39	17.38	8.06	37.49	<0.001
DN_Indoor*	-2.79	0.51	0.061	0.02	0.17	<0.001
DN_Outdoor*	-1.86	0.51	0.16	0.06	0.42	<0.001
Hand_Indoor*	-2.78	0.58	0.06	0.02	0.20	<0.001
Hand_Outdoor*	-3.15	0.55	0.04	0.01	0.13	<0.001
LT*	-1.28	0.54	0.29	0.09	0.81	<0.05
ST*	-6.60	0.50	0.00	0.00	0.00	<0.001
Sergentomyia spp.**	-1.03	0.34	0.36	0.18	0.69	<0.001

* reference to CBTC, **reference to *Phlebotomus* spp.

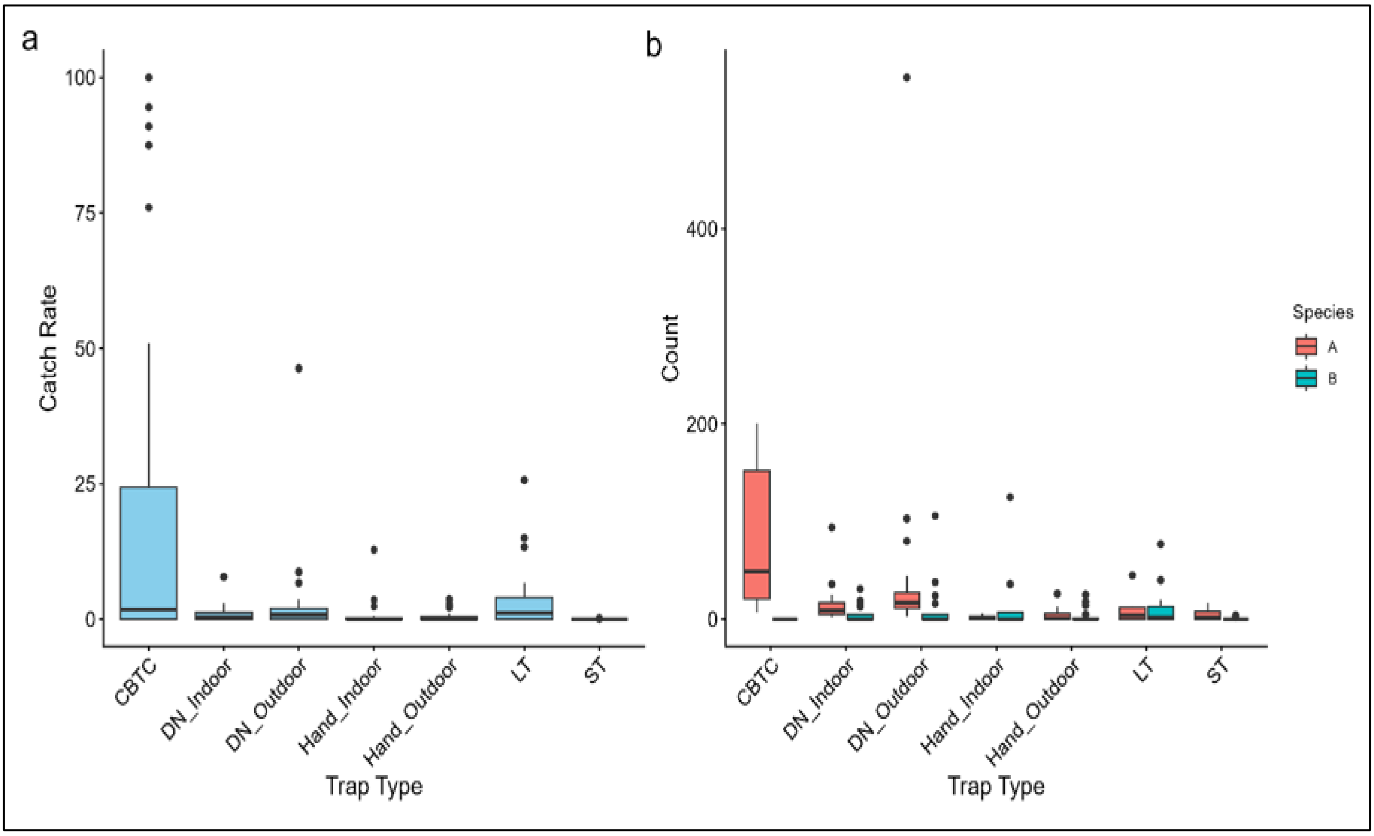

Fig 1 Total Catch Rates of Each Trap Type (a) and Total Catch Counts of Two Sandfly Species in Each Trap Type (b). CBTC – Cattle Baited Trap Collection, DN – Double Net Trap, LT – Light Trap, ST – Sticky Trap. Species A is Phlebotomus spp. and Species B is Sergentomyia spp.

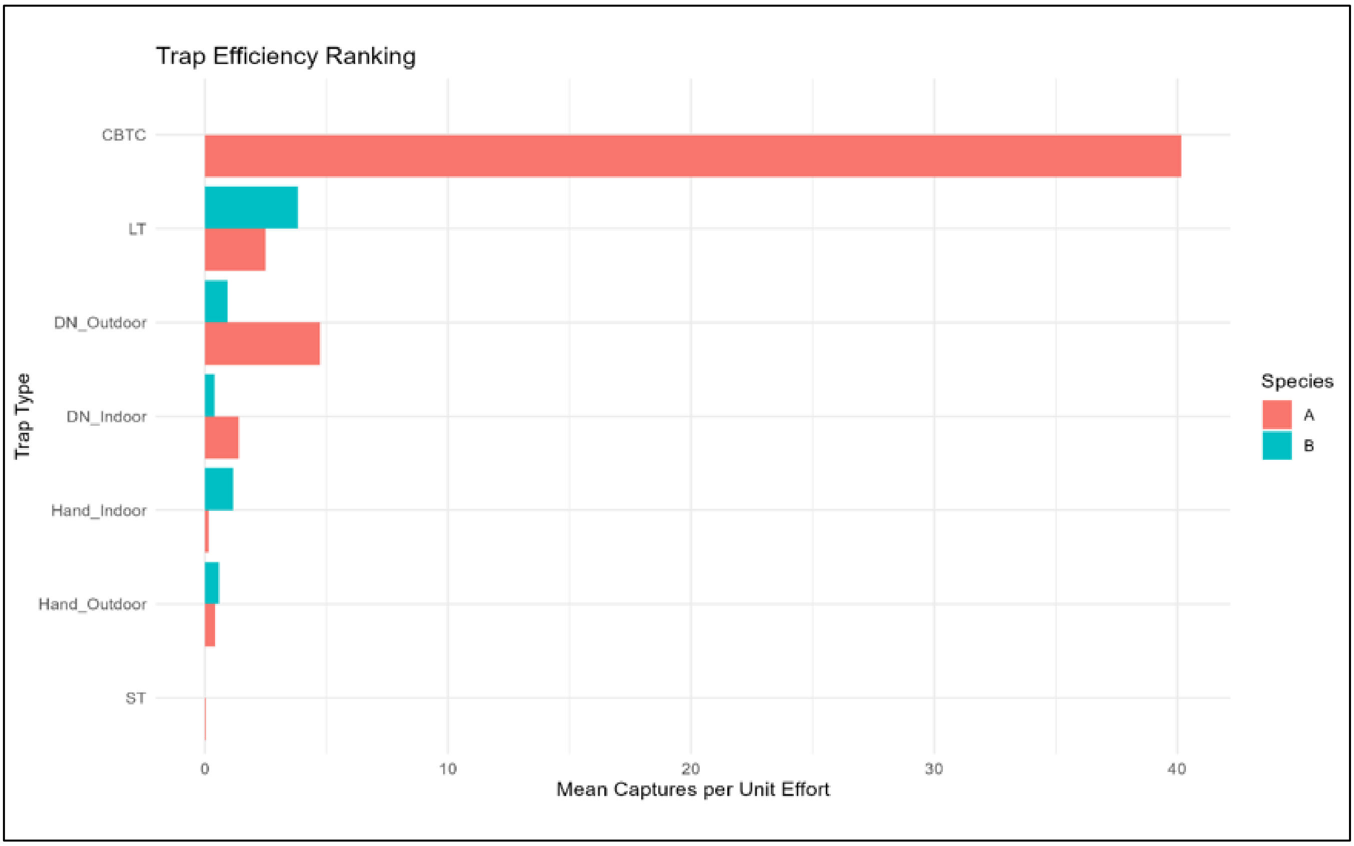

Fig 2 Trap Efficiency Rankings According to Mean Capture per Unit Effort of Each Trap Type. CBTC – Cattle Baited Trap Collection, DN – Double Net Trap, LT – Light Trap, ST – Sticky Trap. Species A is Phlebotomus spp. and Species B is Sergentomyia spp.

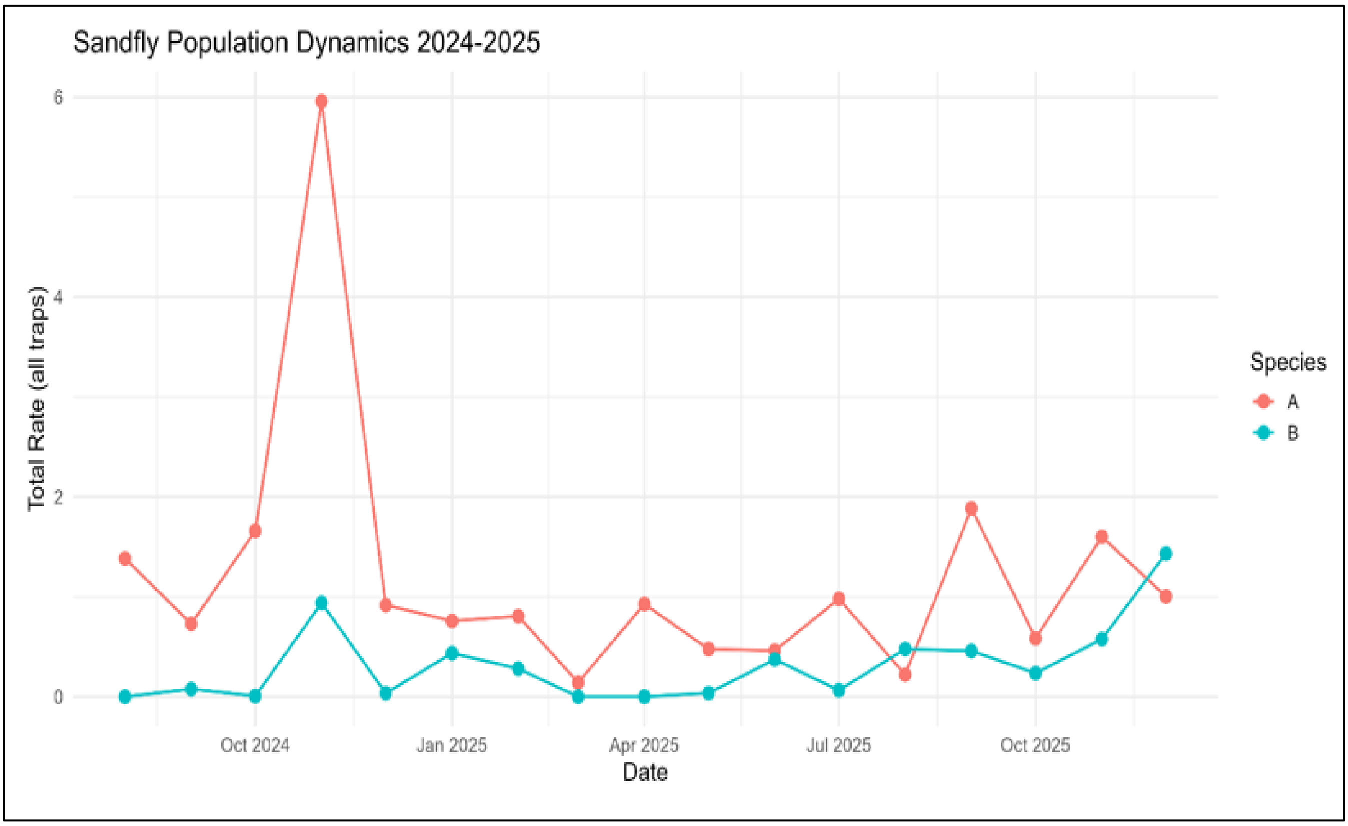

Fig 3 Monthly Variation of Total Collection Rates of Sandfly Species from all Trap Types. Species A is Phlebotomus spp. and Species B is Sergentomyia spp.