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PRELIMINARY STUDY OF THE EFFECTIVENESS OF A STERILE INSECT TECHNIQUE (SIT) DEPLOYMENT AGAINST *AEDES ALBOPICTUS* IN A RESIDENTIAL AREA OF ISRAEL

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Abstract The Sterile Insect Technique (SIT) is a mosquito control method that involves releasing large numbers of sterilized mosquito males into the wild. These sterilized males mate with wild females, but the resulting eggs do not hatch. Over time, this disrupts the reproduction of the wild population, leading to a decline in their numbers and even provoking a mosquito suppression process under certain circumstances. Here we present the preliminary results of a SIT pilot project conducted in a residential area of Kohav Yair, a municipality placed in Central Israel, between July-November 2024. Different mosquito releasing strategies were conducted in private gardens of 8 residential urban sites: weekly releases of 1000 males (1TMW), weekly releases of 500 males (5HMW) and biweekly releases of 1000 males (1TMBW), meaning two weeks with a single release per week followed by non-release period of 2 weeks. The goal was to compare the efficacy of each releasing strategy according to the calculation of 2 key entomological parameters: Population Reduction (D) and Induced Sterility (S). On a weekly basis, adult mosquitoes were collected through BG-Mosquitaire traps to evaluate the reduction of specimens in the environment (D), while standard mosquito ovitraps were also used to catch eggs in order to evaluate the percentage of sterility achieved (S) after the periodical male releases. Results show a very significant effect of all 3 types of mosquito releases (1TMW/5HMW/1TMBW) in both entomological parameters compared with control areas (residential gardens where no mosquito releases were conducted). These results emphasize the convenience to introduce this innovative and sustainable mosquito control tool in large residential mosquito management programs, in order to ensure biting nuisance shortening and also reduce the employment of chemical insecticides in the environment.

Key words: Sterile Insect Technique (SIT), mosquitoes, Asian tiger mosquito, Aedes albopictus.

INTRODUCTION

Mosquitoes pose a significant threat to human health globally by transmitting severe diseases, some of which are lethal, making their control crucial for public health. Most mosquito-human interactions occur in urban environments, where mosquitoes thrive close to households. The common tools used to manage mosquito populations are limited. The main methods available to both individuals and authorities are spraying chemicals for adulticiding treatments and draining standing water sources or carrying out larvicidal treatments in breeding sites, as well as the use of various trapping strategies.

Another tool, currently limited in use due to economic constraints, is the Sterile Insect Technique (SIT), a technique that has been used at different scales in multiple locations around the world (Carvalho et al., 2014, Martin-Park, et al., 2022, Balatsos et al., 2021, Bouyer 2024). SIT involves releasing sterile male mosquitoes into the wild to compete with wild males for mating opportunities. This reduces the number of viable offspring and can lead to population suppression. Currently, almost all rearing processes are labor-intensive and tedious. Several processes make the production insufficient and ineffective:

1. *Separating male pupae from females*. This stage is mostly performed by manually filtering the biomass through glass filters, based on shape and size. This process may be repeated several times to ensure high male pupae rates in the filtered biomass. This results in tedious human labor and product loss in two ways: (A) each mechanical manipulation leads to some individual deaths and (B) large male pupae are lost since they may be the same size as female pupae.

   Radiation of pupae. The presence of water remains in the radiation chamber requires special handling and care (Wang et al., 2023) in order to address potential uneven irradiation distribution, resulting in additional time loss and potential harmful impacts on the pupae and its development (Yamada et al., 2019). Irradiated pupae should be at the same age window to maximize same sterility rate across the entire batch (Yamada et al., 2020), adding further complexity and strict rearing scheduling, resulting in more time loss and more required working men-power, strict QA process and management and overall added costs of the entire process.
2. *Packing the sterile males*. Immediately after radiation, pupae are manually placed at the bottom of release bottles and allowed to emerge and sexually mature. Regardless of the sexing method, scaling to millions of mosquitoes per day, or thousands of release canisters, the packaging process becomes by itself a labour intensive process, prone to human errors, and prone to additional damages to the pupa, occupying factory space for about three days, and results in releasing older mosquitoes into the wild. As part of the process, it requires waiting for all pupa to emerge, resulting in having both young and older adults in the same canister, aside for more manual handling. As the males only live about 7-10 days in the wild, it means some half of them would die within just a few days, resulting in SIT programs releasing mosquitoes twice a week, which has a great impact on logistics, area coverage, and eventually costs.

The above points, covering the packaging of mosquitoes, irradiation and sexing, all three together, were the key factors preventing SIT becoming widely adopted with affordable pricing to municipalities and private houses.

In this study we solved these production-related issues and made the process efficient from rearing through release logistics by implementing automation and new rearing methods, with a single flow process with minimal human intervention (e.g. manual packaging is eliminated). This reduces human involvement to the bare minimum - production of 1M sterile males ready for release plus the required males and females for colony maintenance, requires only a single worker. This makes the production by far the most efficient.

Automation became possible by implementing a novel AI-based sex sorter and packing machine into the rearing process. It processes hundreds of adult mosquitoes per second and supports colony maintenance. After processing, sorted individuals are packed in small containers that can be easily irradiated without water interference. Manipulation at the adult stage allows us to benefit from having the strongest and largest males as well as minimize losses and being less prone to human error or very strict rearing protocols with specific irradiation time windows. Immediately after radiation, the containers are stacked and ready for release in the wild without delays - ensuring the released mosquitoes are as young and fresh as possible. The two main advantages of this technology- it can be quickly adapted and implemented into any mosquito rearing facility, of any species. And due to its modularity, production can be easily expanded if needed.

The experiment described in this paper was conducted as a monitoring plan, part of a vast commercial mosquito control program against the Asian tiger mosquito (*Aedes albopictus*). The method was approved by the Israel Ministry of Environmental Protection and, resulted in the first ever commercial program where a pest control company is providing the service in Israel. The global control program ran from June to November 2024, during which an accumulated number of 8.5 million sterile males were released across hundreds of sites in Israel - including public gardens, playgrounds, parks, cemeteries, synagogues, kindergartens, hotels, schools, and private households. In this paper we present the preliminary efficacy data of a specific monitoring program conducted in 10 residential areas included in the mosquito release program. The goal was to obtain quantitative information about 2 key entomological parameters in SIT projects: reduction of tiger mosquito females and egg hatching rates.

MATERIALS AND METHODS

Mosquitoes. All mosquitoes supplied for the SIT program reared in Senecio's colony running for 5 years, with refreshment of WT individuals on monthly basis during mosquito season. Larvae held in rearing trays, in temperature of 29ºC, under 12L:12D light regime and fed with grounded Sera Vipan described by Carvalho et al, 20141. At sixth day of rearing, the larvae and pupae were inserted into the sorting and packing machine with small amount of food and held in the same conditions as the rearing room. The adult mosquitoes eclosed inside the machine.

Locations and treatments. The experiment was set as a monitoring program, and took place in Khohav Yair, located in central Israel. It is a city with approximately 10,000 residents, ranked 9 out of 10 in socio-economic ranking (while 10 is the highest), with majority of private properties.

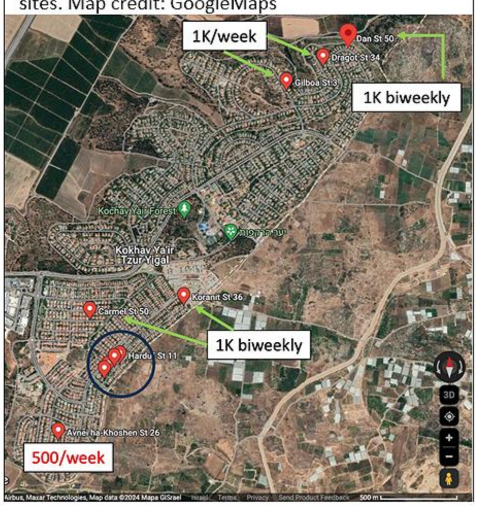

Figure 1a. A map showing all sites in Kohav Yair, Israel. See fig no. 1b for details about the circled sites. Map credit: GoogleMaps

Figure 1b. A map of sites encircled in figure no. 1a. Map credit:

In the experiment participated 10 private households, for almost the entire period. They were divided to four treatment groups: Control (no mosquitoes release at all for the entire period), Release of 1K sterile males weekly (1TMW), Release of 500 sterile males weekly (5HMW), Release of 1K sterile males biweekly (two weeks release, two weeks pause; 1TMBW), Males packing, handling and sterilization. Male mosquitoes were sorted and packaged, into small canisters by batches of 500 and 1K individuals. The canisters were placed in cooling boxes holding temperature of 5.5ºC and stayed there up to the time of release, except half an hour for radiation. Irradiation process typically occurred every morning, and by noon same day, the cooling box with the canisters was collected by a field technician for subsequent release, typically happening the following morning. Releases were performed at the morning hours, between 7:30 - 10:30AM. Five minutes before their release, the canisters were taken out of the box to allow the mosquitoes to recover from cooling prior their release.

Traps. At all participating households, two adult traps and one oviposition trap were placed from July 24th to November 6th of 2024. Visits to collect adults and eggs recorded in those traps were done in a weekly basis during the study period. Adult trap employed was BG-Mosquitaire, equipped with BG-Sweetscent lure and mesh basket for collection of the trapped adults. For oviposition, In2Care Mosquito Trap (without the guaze floater), covered with masking tape serving as oviposition surface, and filled with tap water covering the tape. Both trap types were positioned in wind protected and fully shaded spots in the households' gardens, usually under trees and bushes, with minimal distance of 5 meters between them. If there was not enough shade and protection from wind, they were placed adjacent to walls, with minimal disturbance to the residents. Upon mosquitoes release, the adult mesh collection baskets and the oviposition trap were tagged with their address, and replaced with clean ones, then brought to the lab.

Adult counting. All collection baskets were put in -23ºC freezer for at least 12 hours, then the entire content was counted, with an emphasis of male and female individuals of Ae. albopictus. The rest of mosquitoes caught were classified as 'others' with division to male and female, but without association to specific species.

Fig 2. A scheme of adult counting process

Fig 3. An example of eggs photographed

Eggs trapping, handling and hatching. In a weekly basis the oviposition traps were collected, and replaced with new ones. The collected traps were held in room temperature to period of 4 and up to 6 days, to allow the collected eggs to mature. At the evening before hatching, hatching solution was prepared (700ml DI water, 0.05gr brewer yeast, 0.25gr nutrient broth) and stood in sealed glass jar under 29ºC. At the following day, eggs were gently brushed with water colors compatible paintbrush onto filteration paper. Pieces of dirt such as leaves, small branches and others were removed and the eggs were moved to 50ml urination cup. About 40ml of hatching solution poured onto the eggs, standing for a period of 40 minutes up to an hour. At the end of this period the contents were poured and filtered with filtration paper disc divided to segments. The disc was placed in a petri dish and clutches of eggs were spread with water dropped with pipette and allowed to dry for 24 hours. After drying, the filtration disc segments were photographed and the eggs were tagged by hatched and non hatched using annotation program.

Then, the hatching rate was calculated for each address. Public perception indicators. Qualitative survey about discomfort due to Ae albopictus activity before and after treatments (males releases) was carried out during the study. A total of 70 families participated in the survey, scoring from 1-5 the biting perception on their gardens both before and after the treatments, where 5 represents the worst situation, being almost unable to sit in the garden, and 1 represents almost none biting events.

RESULTS AND DISCUSSION

The results obtained in this study were based solely on those parameters that we were able to compare in all study areas for each capture or eggs count date, from the beginning of August, until the end of November 2024, discarding those that did not coincide in order to obtain greater statistical rigor, although the control plan covers a broader time period.

Quantitative Indicators

Regarding % hatching, the results were: 5HMW (mean = 28.25%, median = 28.5%) and 1TMBW (mean = 28.98%, median = 18.0%), while 1TMW has a mean of 37.44% and Control a mean of 34.15%. These two first treatments had similar and considerably lower average hatch rates than the other three treatments. Both treatments show similar average hatch rates, but the 1TMBW treatment has a considerably lower median (18%), indicating that half of the observations for this treatment were below that value. This suggests that overall, 1TMBW might be slightly more effective, as it reduces the hatch rate in most cases while allowing a lower number of site visits. While both treatments are effective, 1TMBW is likely the more effective treatment in reducing egg hatch rate, considering both the lower median and larger sample size. In addition, based on an extended graphical analysis for all the entire period of treatments and R-squared values, the 5HMW treatment appears to be the most effective in reducing hatching percentage. Although all treatments show a downward trend, 5HMW has the lowest average hatching percentage and a moderate R-squared value, suggesting a consistent reduction in hatching rate over time. During almost the entire study period, except for two specific periods for the 1TMW, and at the beginning of 1TMBW and 5HMW treatments, the % Hatching graphs remain below the Control area line for all the three treatments.

Figure 4. Percentage hatch rate over time by treatment area

An additional parameter that has been considered in the data analysis is the average number of eggs per sample recorded throughout the successive counts in each treatment area. This data, despite not having a particularly relevant statistical or entomological value (because it can be affected by a large sort of environmental parameters as rainfalls, number and suitability of breeding sites, etc.), shows that, on average, the Control areas recorded an average number of eggs per ovitrap that was much higher than the rest of the areas: Control (147), 1TMW (55.67), 1TMBW (47.33), 5HMW (37). This parameter, although it should be analyzed in greater depth in successive experiments and may be affected by other environmental parameters, can also give an indication of the effectiveness of the treatments in reducing oviposition, especially taking into account that it is an average value, and that in the first stages of the study, the values were very similar in all areas. It would be advisable to pay attention to this parameter by replicating the study in different areas and with a greater number of ovitraps. In Control areas with no sterile male releases, egg numbers and hatching rates remained stable and relatively high. In treatment areas, hatching rates fluctuated but showed an overall decrease, with some areas experiencing drops as low as 0-10%. The timing and frequency of sterile male releases appeared to affect the effectiveness of the treatment. Additionally, the proximity of adult traps to ovitraps influenced results, with adult traps potentially attracting wild mosquitoes from neighboring areas.

Running a graph about Time Series for *Ae. albopictus* females by Treatment with LOESS trend (Figure 5), for the different time series it is observed that: in 1TMBW, the total number of females Ae. albopictus fluctuates over time, with a peak near the end of August. The LOESS trend line shows a general downward trend after the peak.; in 1TMW, the total number of females also fluctuates over time, with a peak in mid-August. The LOESS trend line shows a general downward trend after the peak; in 5HMW, the total number of females fluctuates over time, with a peak near the end of October. The LOESS trend line shows a general downward trend after the peak; in Control, the total number of females has a smaller fluctuation over time, with a peak near the end of August and another smaller peak at the end of October. The LOESS trend line mostly follows the peaks and valleys of the actual data. All treatments show a general downward trend after the initial peak(s), although the shape of the trend varies slightly between treatments and has not relevance in the Control area.

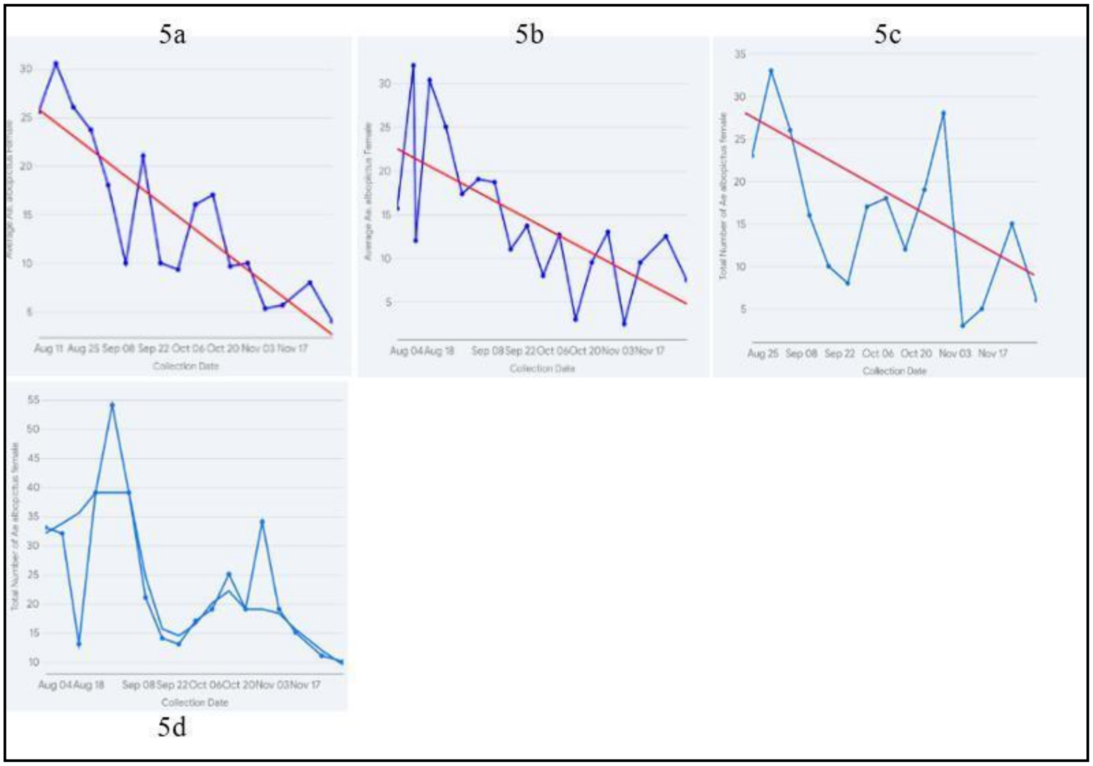

Figure 5. Time Series for Ae albopictus female Over Time by Treatment Area (5a: 1TMBW; 5b: 1TMW; 5c: 5HMW; 5d: Control); Note that scales are not the same as in three treatment groups (lower) as in Control group (higher).

The fluctuations in the number of female *Ae. albopictus* captured over time could be due to several factors, such as seasonal variation: mosquito populations often fluctuate seasonally due to changes in temperature, rainfall, and breeding site availability, humidity and the availability of host plants, could also affect mosquito abundance, or also random events, such as predation or competition with other species. To rule out some environmental parameters such as rainfall, data from nearby meteorological stations have been analyzed, resulting in rainfall of less than 18 L/m2 for a period at the end of September, and more abundant (greater than 40 L/m2) in the month of December, that is, outside the study period. The average temperature throughout the entire study period was around 30ºC, without significant oscillations that could affect the behavior of the mosquitoes. Nevertheless, the different treatments may also have a key role and effect on the mosquito population dynamics, although further analysis is needed to determine the significance of any observed differences.

Results are based on all surveys carried out on the participating population in the SIT pilot program of central region of Israel. A total of 70 families participated and the houses included in the survey represented a mix of private houses or a first floor apartment with a garden. The majority of the gardens were between 50-200 square meters of total area. Most of the houses were between other houses, such that their garden borders with the neighbor's garden. The results of the qualitative perception of the participants show that the feeling of the residents shifted as seen on the graph from majority of the residents starting a marking of 4-5 to majority of the families ranking the results with 1-2 marking. The average ranking for sitting outside prior start of the program was 4.16, and the average ranking at the last treatment was 2. A clear indication of the satisfaction of the residents.

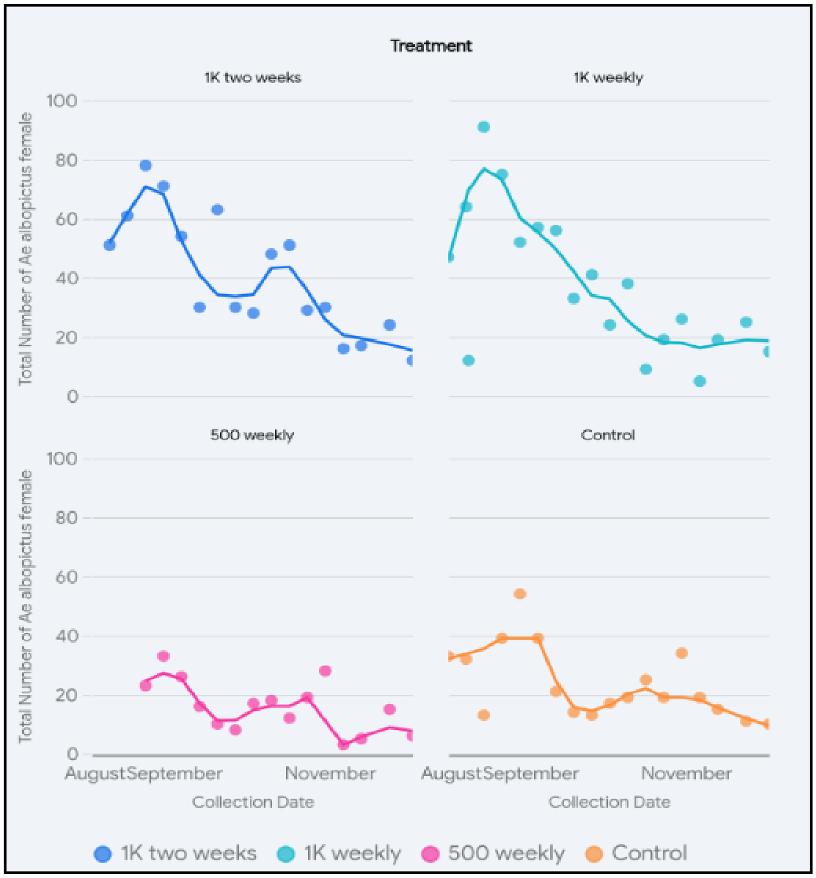

Figure 6. Time Series for *Ae. albopictus* female Over Time by Treatment Area; Comparison at the same scale.

Qualitative Indicators

Results are based on all surveys carried out on the participating population in the SIT pilot program of central region of Israel. A total of 70 families participated and the houses included in the survey represented a mix of private houses or a first floor apartment with a garden. The majority of the gardens were between 50-200 square meters of total area. Most of the houses were between other houses, such that their garden borders with the neighbor's garden. The results of the qualitative perception of the participants show that the feeling of the residents shifted as seen on the graph from majority of the residents starting a marking of 4-5 to majority of the families ranking the results with 1-2 marking. The average ranking for sitting outside prior start of the program was 4.16, and the average ranking at the last treatment was 2. A clear indication of the satisfaction of the residents.

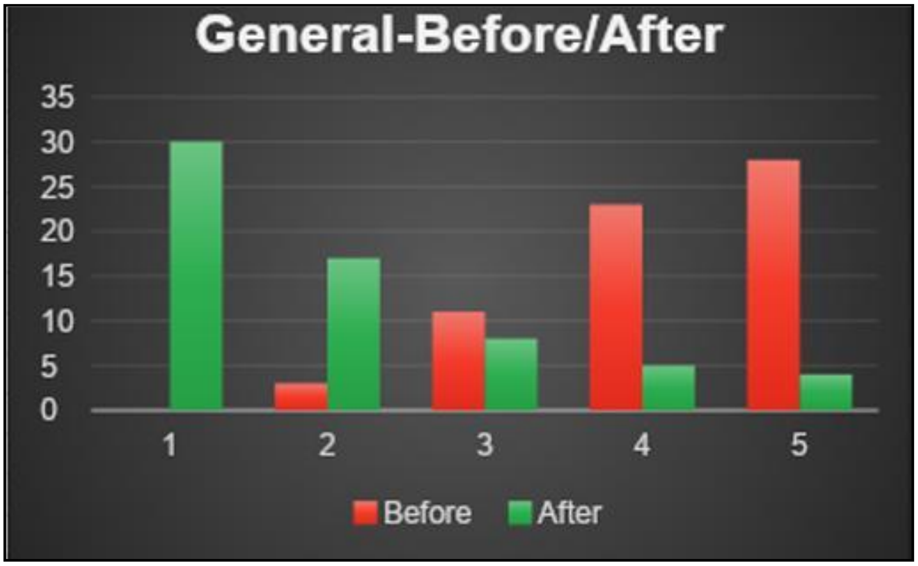

Figure 7. Comparison of qualitative perception of discomfort due to Ae albopictus before and after treatments. 1-5 shows the bitting perception on their gardens before (red) and after (green) the treatments, where 5 represents the worst situation, being almost unable to sit in the garden, and 1 represents almost none biting events.

CONCLUSIONS

First of all, we must take into consideration that this field study is included within a real control plan for wild tiger mosquito populations in private residences, in which other mosquito control tools have not been used, so the effectiveness of this technique should be substantially improved if it had been used in combination with other tools framed in an integrated mosquito management approach9. In these terms, we must consider two types of parameters to assess, those purely quantitative (number of captures of adult insects, % hatching) and those qualitative (perception of residents).

Regarding the quantitative data analyzed, from the database collected during the treatment period, we focused on two key parameters: the hatching rate of Ae. albopictus eggs as an indicator of the induction of sterility due to the different types of treatment; and secondly, the number of captures of Ae. albopictus females in the BG traps located in the different treatment areas as an indicator of a possible population decline of adult insects due to treatments.

If we only pay attention to the % hatching, the most effective treatments, i.e. those with the lowest average hatch rates, were: 5HMW (28.25%) and 1TMBW (28.98%), while 1TMW has a higher mean of 37.44% and Control a mean of 34.15%. These two first treatments had similar and considerably lower average hatch rates than the other three treatments. Both treatments show similar average hatch rates, but the 1TMBW treatment has a considerably lower median (18%), indicating that half of the observations for this treatment were below that value.

This suggests that overall, 1TMBW might be slightly more effective, as it reduces the hatch rate in most cases. While both treatments are effective, 1TMBW is likely the more effective treatment in reducing egg hatch rate, considering both the lower median and larger sample size. Additionally, the average egg count per ovitrap also obtained the lowest average results in 1TMBW and 5HMW, despite the high initial figures for each of the treatment groups.

Regarding adult female captures, the 1TMW and 1TMBW treatments show very marked descending trend lines, which could indicate a good effectiveness of the sterile male release treatments in reducing the population of *Ae. albopictus* over time, especially considering that in these two treatment areas, mosquito populations were initially higher than in the other. If we compare the trend lines of captures of adult females in the four areas, the Control areas show greater stability than the rest of the areas in the number of captures over time. Based on the data provided by the captured females, the 5HMW treatment appears to be the most effective in reducing the number of females of *Ae. albopictus*. It has the lowest total sum, the lowest mean and an overall downward trend. We must consider that the other two treatments were very effective in reducing the initial population, with a marked downward trend line, so we assume that the impact of the treatments was decisive in this decline. However, it is important to note that all treatments show considerable fluctuations, and the sample size is relatively small. The environmental parameters during the study period were stable in terms of temperature and precipitation (very scarce, practically irrelevant), which rules out interference of these variables in the study results.

If we consider the purely qualitative aspects, such as the residents' perception of the effectiveness of the treatments (reduction of discomfort from bites and detected activity of tiger mosquitoes in the outdoor areas of their homes) we can assure that all the treatments had a very positive impact. The general impressions of the participants were unanimous: they perceived a significant reduction in mosquito populations and biting rates in areas where sterile males were released. Residents reported high satisfaction with the service, noting a dramatic decrease in mosquito annoyance. The results also suggest that the timing and location of sterile male releases are crucial for optimal effectiveness.

Overall, the results obtained from the analysis of qualitative data suggest that the most effective and promising treatments would be 1TMBW and 5HMW, although to give statistical robustness to these models it would be necessary to extend the study for a longer period of time and carry out more observations in different locations including also higher number of traps. We assume, as a hypothesis, that with a replication of the different proposed models on a larger scale, the results of 1TMW and 1TMBW could present a small variation in results, both being effective in residential areas. Finally, it is important to emphasize that the successful SIT application could led to relevant improvements in pest control, minimizing the need for chemical interventions and reducing associated environmental risks.

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