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Comparative Attractiveness of Fermented Fruit-Based Baits for *Aedes Aegypti* and *Culex Quinquefasciatus*

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ABSTRACT

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Mosquitoes are important vectors of several diseases, and the development of effective, low cost, and locally available attractants can support mosquito surveillance and management. The present study evaluated the attractiveness of fermented fruit-based baits to *Aedes aegypti* and *Culex quinquefasciatus* under controlled laboratory conditions. Twelve fruit treatments, including guava, Chaunsa mango, Sindhri mango, Langra mango, Anwar Ratol mango, plum, watermelon, muskmelon, orange, pineapple, peach, and banana, were evaluated against a constant sugar only control. Each fruit treatment was prepared using fruit pulp and baker's yeast, while the control contained sugar only. The experiment was conducted in three phases: prescreening, screening, and post screening. In the prescreening and screening phases, mosquito attraction was recorded at 10-minute intervals for one hour. Based on their observed performance, guava, plum, and banana were selected for post screening against the sugar only control. Each experimental cage contained 40 mosquitoes, comprising 20 males and 20 females, and the relative attraction was calculated from the number of mosquitoes attracted to the test bait and control. Among the tested treatments, guava showed the highest observed mean relative attraction in both mosquito species, followed by plum and banana, whereas the sugar only control showed comparatively lower attraction. In the post screening phase, guava again recorded the highest observed mean relative attraction, followed by plum and banana, for both *Aedes aegypti* and *Culex quinquefasciatus*. These findings indicate that fermented fruit-based baits, particularly guava, plum, and banana, have potential as attractant formulations for mosquito surveillance. However, CO₂ production and volatile compounds were not directly measured in the present study; therefore, the specific mechanisms responsible for the observed attraction cannot be confirmed. Further studies involving independent replication, chemical characterization, optimization of fermentation conditions, and field evaluation are recommended to determine the reliability and practical applicability of these baits.

Introduction

Mosquitoes have been among the most dangerous disease vectors in human history, responsible for transmitting a wide range of illnesses that continue to affect public health, economic stability, and social wellbeing in both developing and developed nations. These small insects, often seen as mere nuisances, carry pathogens that lead to diseases such as malaria, dengue fever, Zika virus, yellow fever, chikungunya, and West Nile virus. According to the World Health Organization (WHO), vector borne diseases account for over 17% of all infectious diseases globally and cause more than 700,000 deaths annually (World Health Organization, 2023). Among all vectors, mosquitoes (family: Culicidae) are considered the most significant. Their global distribution, adaptability to various climates, and complex life cycle allow them to thrive in diverse habitats ranging from urban areas to agricultural fields. The three major mosquito genera involved in disease transmission are *Anopheles*, *Aedes*, and *Culex*. Each genus is associated with specific diseases, with *Anopheles* associated with malaria, *Aedes* with dengue, Zika, and chikungunya, and *Culex* with Japanese encephalitis and filariasis (Service, 1993).

In recent decades, mosquito borne diseases have expanded due to several anthropogenic and environmental factors. Climate change, unplanned urbanization, poor sanitation, global trade, and human migration have contributed to the proliferation of mosquitoes in previously non endemic areas. Rising temperatures and increased rainfall provide favorable conditions for mosquito breeding and larval development, while globalization has facilitated the movement of both mosquitoes and the pathogens they carry (Weaver & Reisen, 2010). Dengue fever, transmitted primarily by *Aedes aegypti*, has emerged as one of the important mosquito borne diseases in the world. The global incidence of dengue has increased substantially over the past several decades, with an estimated 390 million dengue infections occurring annually, of which approximately 96 million manifests clinically (Bhatt et al., 2013). Dengue is now endemic in more than 100 countries, including Pakistan, India, Bangladesh, Brazil, and the Philippines.

In the South Asian context, particularly in Pakistan, mosquito borne diseases pose a significant and recurring threat. Over the last decade, Pakistan has experienced multiple dengue outbreaks, with thousands of reported cases annually in urban centers such as Lahore, Karachi, and Rawalpindi. According to the Punjab Health Department, more than 52,000 dengue cases and over 200 deaths were reported during the 2011 outbreak alone (Ali et al., 2012).

Apart from dengue, Pakistan is also vulnerable to other mosquito borne diseases. However, the epidemiological importance of mosquito species varies according to their ecological characteristics and geographical distribution. One of the key factors that complicates mosquito control is the behavioral adaptation of vector species. Mosquitoes exhibit species specific feeding preferences, resting habits, and host seeking behaviors. For example, *Aedes aegypti* tends to bite during the daytime and is commonly associated with human habitation, whereas *Culex* species are generally more active during the night and can utilize polluted and organically enriched water bodies for breeding. These ecological adaptations highlight the importance of understanding species specific behavior when developing mosquito surveillance and management strategies (Takken & Knols, 1999).

In this regard, understanding the host seeking behavior of mosquitoes is vital. Mosquitoes use a combination of visual, thermal, and chemical cues to locate their hosts. Among these cues, carbon dioxide (CO₂) plays an important role. It serves as a long range attractant, alerting the mosquito to the presence of a vertebrate host. Upon sensing CO₂, mosquitoes can engage in anemotactic flight, flying upwind toward the odor source, followed by close range attraction using other chemical cues such as lactic acid, ammonia, and skin volatiles (McMeniman et al., 2014). The importance of CO₂ in mosquito attraction has been extensively documented. CO₂ is released with each breath we exhale, making it an important cue for blood feeding insects.

Research has shown that mosquitoes can detect increases in ambient CO₂ concentrations and use this gradient to locate potential hosts. Consequently, traps incorporating CO₂ from sources such as dry ice, yeast fermentation, or compressed gas can enhance mosquito attraction compared with traps relying solely on other cues (Dekker et al., 2005).

However, despite its efficacy, the use of CO₂ in mosquito surveillance and control can be limited by cost and logistics. Traditional sources of CO₂, such as dry ice and compressed gas cylinders, are not always feasible in field conditions, especially in resource limited settings. Dry ice requires appropriate storage and transportation, while gas cylinders require suitable equipment and regular supply. These factors can create challenges for effective vector monitoring in areas where resources and specialized equipment are limited (Burkett et al., 2001). In response, researchers have investigated alternative approaches that can provide mosquito attracting cues through low cost and easily accessible materials. One such method involves yeast fermentation to generate CO₂. This technique involves fermentation of sugar containing substrates by yeast, resulting in the production of carbon dioxide and ethanol. Studies have demonstrated the potential of yeast generated CO₂ as an alternative source for mosquito sampling (Saitoh et al., 2004).

More recently, attention has shifted toward the use of fermented fruit substrates for mosquito attraction. Fruit materials naturally contain sugars, water, organic acids, and other compounds that can support fermentation. When inoculated with

baker's yeast, fruit substrates can undergo fermentation and produce CO₂ and other fermentation associated volatile compounds. These compounds may influence mosquito behavior and contribute to the overall attractiveness of the bait. The composition of fruits varies considerably, and differences in sugar content, acidity, moisture, and volatile compounds may influence fermentation and mosquito attraction. Fruit based attractants are particularly relevant because fruits such as guava, mango, plum, watermelon, muskmelon, orange, pineapple, peach, and banana are widely available and relatively inexpensive. Mango is also represented by several commercially important cultivars, including Chaunsa, Sindhri, Langra, and Anwar Ratol. Differences among fruit species and cultivars may influence the characteristics of the resulting fermented bait. Their potential to act as affordable and locally available mosquito attractants makes them worthy of investigation, particularly in resource limited settings.

Mosquitoes possess a highly evolved and specialized olfactory system that enables them to locate potential hosts through chemical cues present in the environment. Among these cues, carbon dioxide is an important long range stimulus. Its importance stems from its biological relevance as a product of respiration by vertebrates, including humans (World Health Organization, 2023). The maxillary palps of female mosquitoes contain sensory structures capable of detecting changes in ambient CO₂ concentrations. Upon detection, mosquitoes can exhibit anemotactic flight, meaning they fly upwind toward the source. This initial detection can contribute to a sequence of behavioral responses, including orientation, approach, and attraction to the source (Service, 1993). CO₂ can also interact with other host associated cues, including lactic acid, ammonia, and skin volatiles, which may influence mosquito behavior at closer distances (McMeniman et al., 2014).

Different mosquito species may respond differently to chemical and environmental cues. *Aedes aegypti* and *Culex quinquefasciatus* differ in their ecology, activity patterns, host seeking behavior, and environmental preferences. These behavioral differences highlight the importance of evaluating mosquito attractants against different species rather than assuming that a particular bait will produce the same response in all mosquitoes. The use of CO₂ in mosquito traps is well established in both academic and operational settings. Commonly used sources include dry ice and compressed CO₂ gas. Dry ice is widely used in entomological studies because of its ability to release CO₂ continuously. However, it requires appropriate storage and transportation and can be difficult to maintain under warm field conditions. Compressed CO₂ gas cylinders can provide controlled delivery but require cylinders, regulators, suitable equipment, and regular access to the gas supply. These limitations have encouraged the investigation of alternative sources and methods for mosquito attraction.

Given the limitations of traditional CO₂ sources, attention has shifted toward biological fermentation methods that use locally available materials. Yeast fermentation can generate CO₂ by converting fermentable sugars into ethanol and CO₂. The general fermentation reaction can be represented as:



Yeast based CO₂ production has been tested successfully in different mosquito sampling systems. Saitoh et al. (2004) demonstrated the potential of yeast generated CO₂ as a convenient source of carbon dioxide for adult mosquito sampling. Similarly, other studies have investigated yeast fermentation as an alternative source of CO₂ for mosquito traps and have demonstrated its potential for enhancing mosquito collection (Smallegange et al., 2010; Nakata et al., 2020). Fruit substrates can provide a natural source of fermentable sugars and other compounds that may support yeast activity. Natural fruits such as guava, mango, plum, orange, muskmelon, watermelon, peach, pineapple, and banana contain varying amounts of sugars and water and can therefore serve as potential substrates for fermentation. When inoculated with baker's yeast, these substrates may undergo fermentation and produce CO₂ together with other volatile compounds. The resulting combination of fermentation products and fruit derived odors may influence mosquito attraction.

The use of locally available fruit substrates may provide several potential advantages. These materials are generally accessible, relatively inexpensive, biodegradable, and easy to prepare. Their use may therefore be relevant for mosquito surveillance in settings where conventional trapping materials or commercial attractants are difficult to obtain. However, the attractiveness of different fruit substrates cannot be assumed to be equal because fruit composition and fermentation characteristics vary among species and cultivars. Comparative laboratory evaluation is therefore necessary to determine which substrates produce greater mosquito attraction under standardized conditions.

Pakistan, with a population exceeding 240 million, is among the countries affected by mosquito borne diseases. Dengue outbreaks have occurred repeatedly, with major epidemics affecting different provinces including Punjab, Khyber Pakhtunkhwa, and Sindh. In 2011, more than 52,000 confirmed dengue cases were reported, alongside hundreds of deaths (Ali et al., 2012).

Several factors complicate mosquito surveillance and vector control in Pakistan. Uncontrolled urbanization can contribute to water stagnation and inadequate drainage, while limited access to entomological equipment can restrict surveillance activities in some areas. The availability and cost of trapping materials can also influence the implementation of mosquito monitoring

programs. Therefore, investigation of simple and locally available materials may provide useful information for developing additional approaches for mosquito surveillance.

Moreover, conventional CO₂ sources may not always be readily available for routine mosquito surveillance. Dry ice requires appropriate storage and transportation, while compressed gas requires specialized equipment and regular supply. Therefore, biological fermentation using locally available materials may provide an alternative approach for generating mosquito attracting cues. Fruit based substrates are particularly interesting because of their availability and their natural sugar content.

The current study was designed to address the need for comparative evaluation of different fermented fruit substrates as mosquito attractants. While previous studies have evaluated yeast fermentation and alternative CO₂ sources, comparatively limited information is available on the relative attractiveness of multiple fermented fruit substrates to both *Aedes aegypti* and *Culex quinquefasciatus* under standardized laboratory conditions.

The study aimed to evaluate the attractiveness of twelve fermented fruit-based baits to *Aedes aegypti* and *Culex quinquefasciatus* under controlled laboratory conditions. It further sought to compare the attractiveness of different fruit substrates and mango cultivars based on the number of mosquitoes attracted, as well as to compare fermented fruit-based treatments with a constant sugar-only control. The study also aimed to identify promising fruit-based treatments through sequential pre-screening and screening bioassays. Based on the screening results, selected treatments, namely **guava, plum, and banana**, were further evaluated against the sugar-only control during the post-screening phase. Finally, the study compared the responses of *Aedes aegypti* and *Culex quinquefasciatus* to the selected fermented fruit-based baits.

Materials And Methods

Study Site

The present study was conducted at the National Entomological Reference Laboratory, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Punjab, Pakistan. The experiment was designed to evaluate the comparative attractiveness of fermented fruit-based baits to two medically important mosquito species, *Aedes aegypti* and *Culex quinquefasciatus*, under controlled laboratory conditions.

The experiment comprised three sequential phases: pre-screening, screening, and post-screening. Twelve fruit-based baits were evaluated along with one control treatment. The control remained constant throughout all three phases and consisted of a sugar-only solution without fruit and without yeast.

Experimental Mosquitoes

Laboratory-maintained cultures of *Aedes aegypti* and *Culex quinquefasciatus* were used in the experiments. Mosquitoes were obtained from the laboratory-maintained culture at the National Entomological Reference Laboratory.

Healthy adult mosquitoes aged 3–5 days were selected for the bioassays. Before each experiment, mosquitoes were deprived of sugar for 24 h to standardize their physiological condition and increase their responsiveness to the tested bait sources.

For each experimental cage, 40 mosquitoes were released, consisting of 20 males and 20 females. The same number and sex ratio were maintained across the experimental treatments.

Insectary Conditions

The mosquito colonies and bioassays were maintained under controlled laboratory conditions. Temperature was maintained at $27 \pm 2^\circ\text{C}$, relative humidity at $70 \pm 5\%$, and the photoperiod was maintained at 12:12 h light: dark.

All bioassays were conducted under the same environmental conditions to minimize variation in mosquito activity and bait attractiveness resulting from environmental factors.

Fruit Materials and Treatment Preparation

Twelve locally available fruits were selected as substrates for the preparation of fermented fruit-based baits. The treatments were designated as follows:

Treatment	Fruit-based bait
T1	Guava
T2	Chaunsa mango
T3	Sindhri mango
T4	Langra mango

T5	Anwar Ratol mango
T6	Plum
T7	Watermelon
T8	Muskmelon
T9	Orange
T10	Pineapple
T11	Peach
T12	Banana
T13	Sugar only (control)

For preparation of each fruit-based bait, 250 g of fresh fruit was used. The fruits were washed thoroughly, peeled where necessary, and cut into small pieces. The fruit material was then blended to obtain a homogeneous pulp. The resulting pulp was passed through a sieve to remove coarse particles and obtain a relatively uniform fruit preparation.

Following preparation of the fruit pulp, 5 g of commercial baker's yeast was added to each fruit-based treatment and thoroughly mixed. The same quantity of yeast was used for each fruit treatment to maintain consistency among the formulations. No additional sugar was added to the fruit-based treatments.

The preparation procedure was followed separately for all 12 fruit substrates.

Control Treatment

The control treatment (T13) consisted of sugar solution only. The required quantity of sugar was dissolved in distilled water to prepare the control solution.

Importantly, no fruit material and no baker's yeast were added to the control treatment. Thus, the control represented a sugar-only treatment and was maintained consistently throughout the pre-screening, screening, and post-screening experiments. The sugar-only control was included in every experimental comparison as a common reference for evaluating the attractiveness of the fermented fruit-based baits.

Preparation of Bait-Soaked Cotton Discs

Cotton discs weighing approximately 0.5 g were used for presenting the bait formulations to the mosquitoes. Each cotton disc was soaked with 5 mL of the respective bait solution. A separate cotton disc was prepared for each fruit-based treatment and for the sugar-only control. The bait-soaked discs were placed in Petri dishes before being introduced into the experimental cages. Equal quantities of bait solution were used for the different treatments to maintain consistency in bait presentation.

Experimental Design

The study consisted of three sequential experimental phases:

1. Pre-screening bioassay
2. Screening bioassay
3. Post-screening bioassay

The pre-screening phase involved individual comparison of each fruit-based bait with the constant sugar-only control. The screening phase involved simultaneous comparison of all 12 fruit-based treatments in groups of three, with the same control included in every cage. Based on performance during the screening phase, Guava, Plum, and Banana were selected for the post-screening phase and compared simultaneously with the sugar-only control.

Pre-screening Bioassay

The pre-screening bioassay was conducted to evaluate the attractiveness of each of the 12 fruit-based baits individually against the constant sugar-only control. A total of 24 experimental cages were used in this phase, comprising 12 cages for *Aedes aegypti* and 12 cages for *Culex quinquefasciatus*. Each fruit-based treatment was evaluated separately for each mosquito species. Therefore, each experimental cage contained:

- One fruit-based bait disc
- One sugar-only control disc

Forty mosquitoes, consisting of 20 males and 20 females, were introduced into each cage. The treatment and control discs were positioned at approximately equal distances from the center of the cage. After introducing the mosquitoes, the number of mosquitoes attracted to each bait was recorded at 10-minute intervals for 1 h. To minimize positional bias, the positions of the treatment and control bait discs were rotated after every 10-minute observation interval.

The pre-screening experiment provided comparative attraction data for all 12 fruit-based baits against the common sugar-only control for both mosquito species.

Screening Bioassay

The screening bioassay was conducted to compare the attractiveness of all 12 fruit-based treatments under simultaneous-choice conditions. A total of eight experimental cages were used, comprising:

- Four cages for *Aedes aegypti*
- Four cages for *Culex quinquefasciatus*

The 12 fruit-based treatments were divided into four groups of three treatments. Each experimental cage contained three fruit-based bait discs and one sugar-only control disc.

Screening Groups for *Aedes aegypti*

The treatment combinations were arranged as follows:

Cage A:

Guava (T1) + Muskmelon (T8) + Sindhri mango (T3) + sugar-only control

Cage B:

Plum (T6) + Anwar Ratol mango (T5) + Pineapple (T10) + sugar-only control

Cage C:

Banana (T12) + Watermelon (T7) + Langra mango (T4) + sugar-only control

Cage D:

Chaunsa mango (T2) + Peach (T11) + Orange (T9) + sugar-only control

Screening Groups for *Culex quinquefasciatus*

The same treatment combinations were used for *Culex quinquefasciatus*:

Cage E:

Guava (T1) + Muskmelon (T8) + Sindhri mango (T3) + sugar-only control

Cage F:

Plum (T6) + Anwar Ratol mango (T5) + Pineapple (T10) + sugar-only control

Cage G:

Banana (T12) + Watermelon (T7) + Langra mango (T4) + sugar-only control

Cage H:

Chaunsa mango (T2) + Peach (T11) + Orange (T9) + sugar-only control

Forty mosquitoes, comprising 20 males and 20 females, were released into each experimental cage. The four bait discs were placed at approximately equal distances from one another. The number of mosquitoes attracted to each bait was recorded at 10-minute intervals for 1 h. The position of each bait disc was rotated after every 10-minute observation interval to reduce the effect of cage position on mosquito attraction. The screening results were used to identify the fruit-based treatments showing the highest attraction for further evaluation in the post-screening phase.

Selection of Treatments for Post-screening

Based on their performance during the screening phase, three fruit-based treatments—Guava (T₁), Plum (T₆), and Banana (T₁₂)—were selected for the post-screening experiment. These treatments showed comparatively higher attraction to mosquitoes during the screening phase and were therefore selected for a final simultaneous comparison. The sugar-only control (T₁₃) was retained as the constant reference treatment.

Post-screening Bioassay

The post-screening bioassay was conducted to provide a final comparative assessment of the three selected fruit-based baits.

Two experimental cages were used in this phase:

- One cage for *Aedes aegypti*
- One cage for *Culex quinquefasciatus*

Each cage contained four bait sources:

- **Guava fermented bait (T₁)**
- **Plum fermented bait (T₆)**
- **Banana fermented bait (T₁₂)**
- **Sugar-only control (T₁₃)**

Forty mosquitoes, comprising 20 males and 20 females, of the respective species were introduced into each cage. The four bait discs were positioned at approximately equal distances from one another. The number of mosquitoes attracted to each bait was recorded at 10-minute intervals for 1 h. The positions of the four bait discs were rotated after every 10-minute observation interval to minimize positional bias. The post-screening experiment provided a direct comparison of the three selected fruit-based baits with the constant sugar-only control.

Observation of Mosquito Attraction

Mosquito attraction was assessed by recording the number of mosquitoes attracted to each bait source during the observation period.

Observations were made at 10-minute intervals over a total period of 60 min. At each observation interval, the number of mosquitoes attracted to each bait was recorded separately. Both male and female mosquitoes were included in the observations. The data were recorded treatment-wise along with the mosquito species, experimental phase, cage identification, observation interval, and number of mosquitoes attracted. The recorded observations were subsequently compiled in Microsoft Excel for data organization and analysis.

Calculation of Relative Attraction

The relative attraction was calculated to determine the attractiveness of each test bait compared with the sugar only control. The number of mosquitoes attracted to the test bait was compared with the number attracted to the control bait. The relative attraction was calculated using the following formula:

$$\text{Relative Attraction (\%)} = \frac{\text{No. of mosquitoes on test bait} - \text{No. of mosquitoes on control}}{\text{No. of mosquitoes on test bait} + \text{No. of mosquitoes on control}} \times 100$$

Data Recording and Management

Following each experimental observation, the data were entered into a master data sheet. The recorded information included:

1. Date of experiment
2. Mosquito species
3. Treatment code

4. Fruit type
5. Experimental phase
6. Cage identification
7. Observation interval
8. Number of mosquitoes attracted
9. Control response
10. Any unusual observations during the experiment

The data were organized in Microsoft Excel before statistical analysis. The observations were subsequently summarized according to treatment and mosquito species.

Statistical Analysis

The experimental data were initially organized and summarized using Microsoft Excel. The mean attraction values and standard errors were calculated for the tested treatments based on the recorded observations. The data were summarized separately for each mosquito species and experimental phase. Graphical representations of treatment responses were prepared using Microsoft Excel and R Software version 4.5.0.

Results

Prescreening Bioassay of *Aedes aegypti*

In the prescreening bioassay, differences were observed in the relative attraction of *Aedes aegypti* to the tested fermented fruit-based baits. Guava showed the highest observed mean attraction, followed by plum and banana, whereas the sugar only control showed the lowest observed attraction among the tested treatments. The remaining fruit-based baits showed intermediate levels of attraction.

Table 1: Mean relative attraction (%) of *Aedes aegypti* to different fermented fruit-based baits during the pre-screening bioassay.

Treatment	Mean (%)	SE
Guava	85.21	4.36
Plum	80.05	4.88
Banana	75.12	5.14
Chaunsa Mango	70.38	4.55
Sindhri Mango	68.44	5.29
Langra Mango	65.02	4.41
Anwar Ratol Mango	63.22	4.96
Watermelon	60.11	5.03
Muskmelon	55.00	5.28
Orange	50.01	4.77
Peach	45.67	5.06
Pineapple	40.90	5.24
Control	20.42	3.87

Values are presented as mean and standard error (SE).

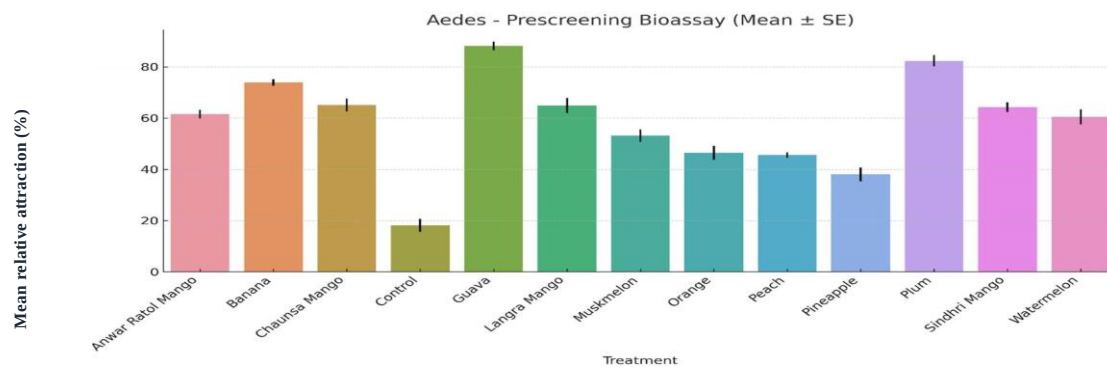


Figure 1: Prescreening bioassay of *Aedes aegypti*.

Prescreening Bioassay of *Culex quinquefasciatus*

In the prescreening bioassay, differences were observed in the relative attraction of *Culex quinquefasciatus* to the tested fermented fruit-based baits. Guava showed the highest observed mean attraction, followed by plum and banana, whereas the sugar only control showed the lowest observed attraction. The remaining fruit-based baits showed intermediate levels of attraction.

Table 2: Mean relative attraction (%) of *Culex quinquefasciatus* to different fermented fruit based baits during the pre-screening bioassay.

Treatment	Mean (%)	SE
Guava	60.22	4.75
Plum	55.43	4.83
Banana	50.89	4.92
Chaunsa Mango	48.01	4.62
Sindhri Mango	45.12	4.68
Langra Mango	42.38	4.81
Anwar Ratol Mango	40.24	4.75
Watermelon	38.55	4.65
Muskmelon	35.33	4.59
Orange	30.78	4.40
Peach	28.61	4.52
Pineapple	25.89	4.67
Control	10.23	3.44

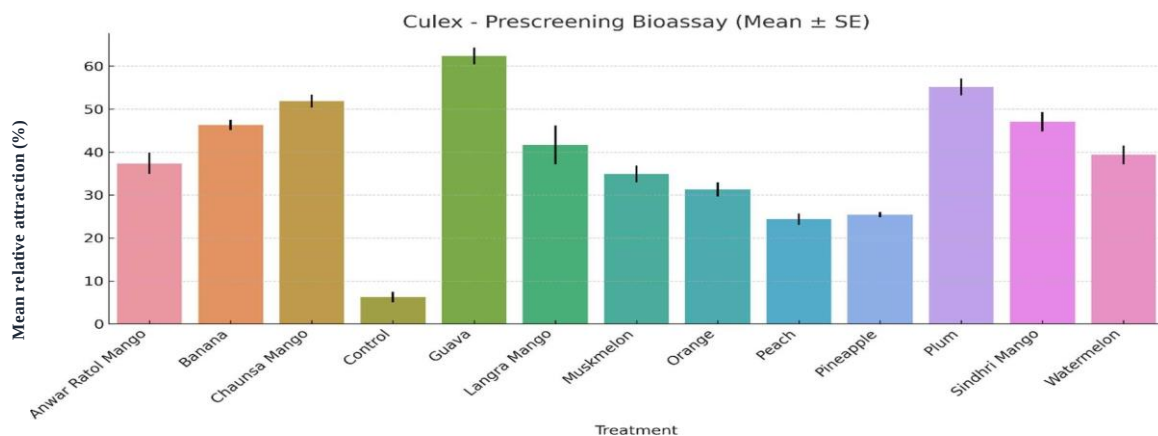


Figure 2. Prescreening bioassay of *Culex quinquefasciatus*.

Screening Bioassay of *Aedes aegypti*

The screening bioassay evaluated all twelve fermented fruit-based treatments in four groups of three treatments, with the sugar only control included in each experimental cage. Guava showed the highest observed mean attraction, followed by plum and banana. The other fruit-based treatments showed varying levels of attraction, while the sugar only control showed the lowest observed attraction.

Table 3: Mean relative attraction (%) of *Aedes aegypti* to different fermented fruit based baits during the screening bioassay.

Treatment	Mean (%)	SE
Guava	84.55	4.63
Plum	80.21	4.92
Banana	75.62	5.11
Chaunsa Mango	70.04	4.88
Sindhri Mango	68.12	5.01
Langra Mango	65.48	4.73
Anwar Ratol Mango	63.09	4.91
Watermelon	60.47	4.85
Muskmelon	55.11	4.98
Orange	50.08	4.72
Peach	45.84	4.99
Pineapple	40.76	4.88
Control	20.45	3.89

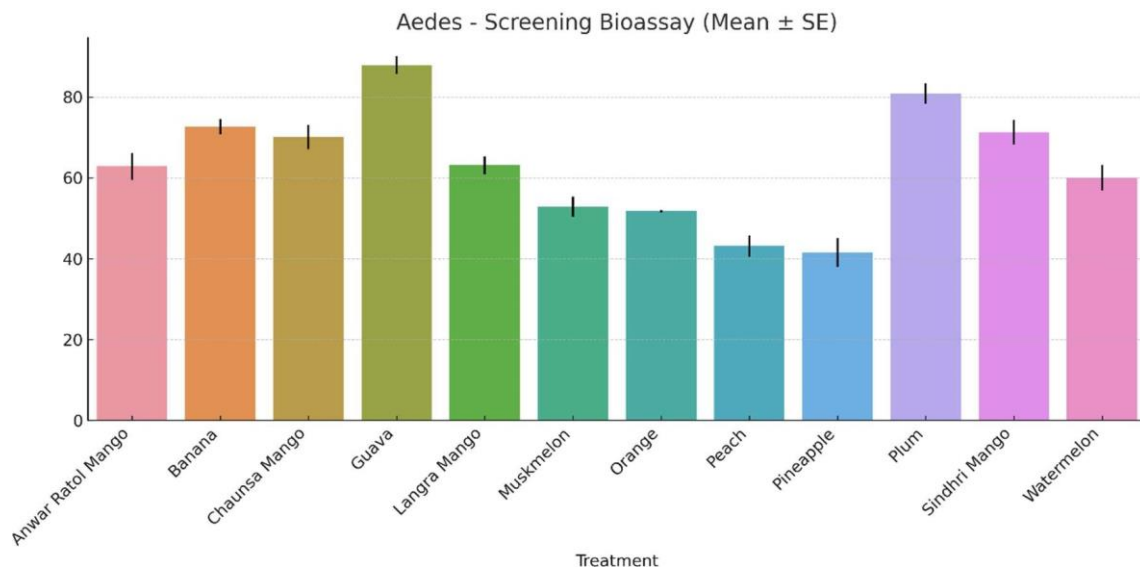
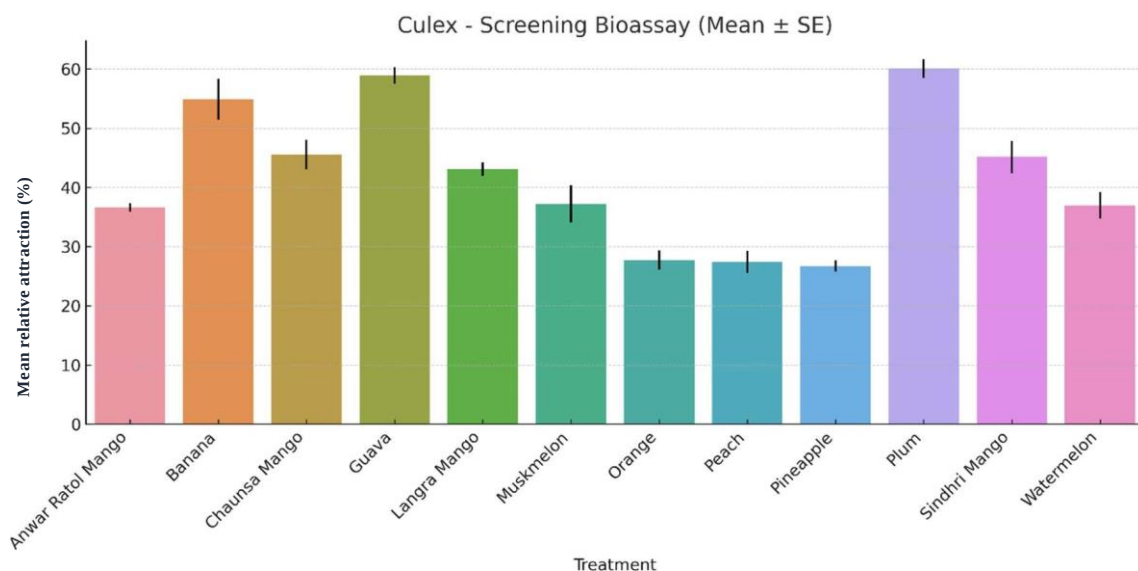


Figure 3. Screening bioassay of *Aedes aegypti*. Screening Bioassay of *Culex quinquefasciatus*

The screening bioassay of *Culex quinquefasciatus* showed variation in the observed relative attraction among the fermented fruit-based treatments. Guava showed the highest observed mean attraction, followed by plum and banana, while the sugar only control showed the lowest observed attraction. The remaining treatments showed intermediate levels of attraction.

Table 4: Mean relative attraction (%) of *Culex quinquefasciatus* to different fermented fruit based baits during the screening bioassay.

Treatment	Mean (%)	SE
Guava	59.42	4.70
Plum	55.33	4.95
Banana	50.91	4.83
Chaunsa Mango	47.84	4.68
Sindhri Mango	45.27	4.76
Langra Mango	42.53	4.66
Anwar Ratol Mango	40.05	4.79
Watermelon	38.44	4.67
Muskmelon	35.01	4.55
Orange	30.62	4.40
Peach	28.21	4.52
Pineapple	25.44	4.63
Control	10.23	3.44

**Figure 4. Screening bioassay of *Culex quinquefasciatus*.****Post screening Bioassay of *Aedes aegypti***

In the post screening bioassay, guava showed the highest observed mean relative attraction among the three selected fermented fruit-based baits, followed by plum and banana. The sugar only control showed the lowest observed attraction. These results were consistent with the general pattern observed during the prescreening and screening phases.

Table 5: Mean relative attraction (%) of *Aedes aegypti* to selected fermented fruit based baits during the post screening bioassay.

Treatment	Mean	SE
Guava	85.12	4.68
Plum	80.44	5.12
Banana	75.39	5.01
Control	20.67	3.80

Values are presented as mean and standard error (SE).

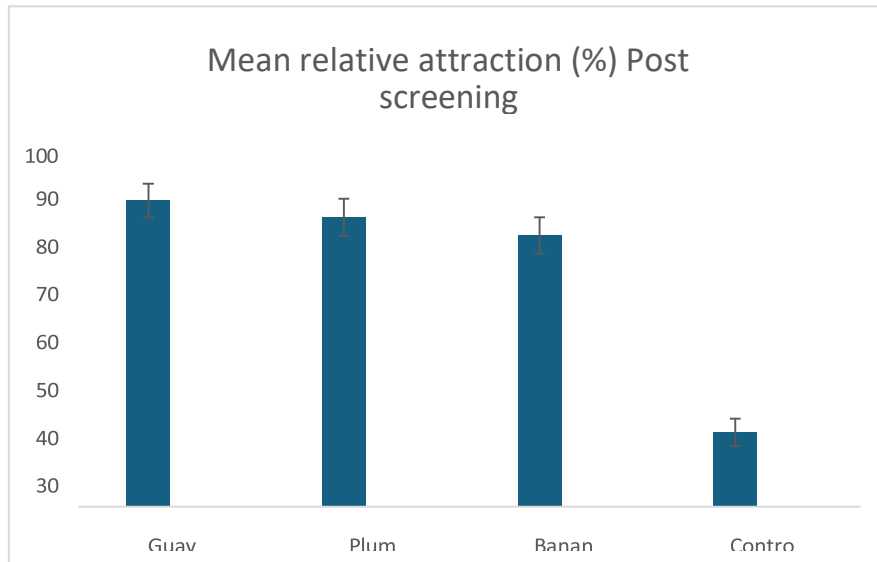


Figure 5. Post screening bioassay of *Aedes aegypti*.

Post screening Bioassay of *Culex quinquefasciatus*

In the post screening bioassay, guava showed the highest observed mean relative attraction among the three selected fermented fruit-based baits, followed by plum and banana. The sugar only control showed the lowest observed attraction. The observed pattern was consistent with the results obtained during the earlier experimental phases.

Table 6: Mean relative attraction (%) of *Culex quinquefasciatus* to selected fermented fruit-based baits during the post screening bioassay.

Treatment	Mean (%)	SE
Guava	60.01	4.70
Plum	55.33	4.95
Banana	50.11	4.83
Control	10.23	3.44

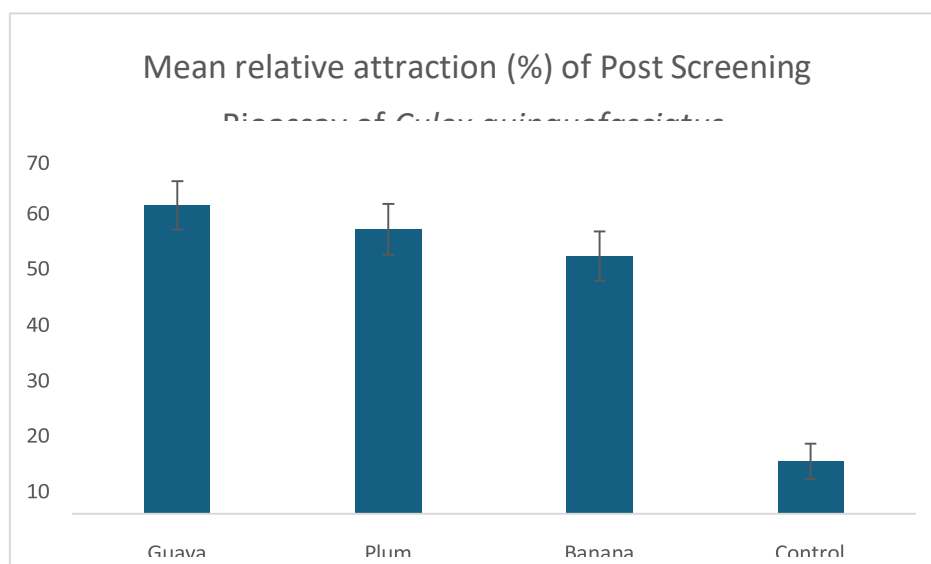


Figure 6: Post screening bioassay of *Culex quinquefasciatus*.

Discussion

This study was conducted to evaluate and compare the attractiveness of different fermented fruit-based baits to two medically important mosquito species, *Aedes aegypti* and *Culex quinquefasciatus*. The primary objective was to determine whether fermented fruit substrates could provide attractive bait sources under controlled laboratory conditions. The results showed variation in mosquito attraction among the tested fruit-based treatments, with guava showing the highest observed attraction across the experimental phases. The observed differences may be related to variation in the chemical composition, sugar content, acidity, moisture, and fermentation characteristics of the different fruit substrates. However, CO₂ emission rates and individual volatile compounds were not directly measured in the present study. Therefore, differences in mosquito attraction cannot be attributed to a specific compound or to differences in CO₂ production alone.

The results clearly demonstrated that the attraction of mosquitoes varied significantly depending on the type of bait used, with guava-based fermenting bait (T1) emerging as the most attractive option across all three experimental phases. This chapter discusses these findings in light of the existing literature, highlights the possible biological mechanisms behind the observed patterns, and explores the practical implications for vector control strategies. During the pre-screening phase, the fermented fruit based baits generally showed higher observed attraction than the sugar only control. Guava showed the highest observed attraction for both mosquito species, followed by plum and banana. The differences among fruit based treatments may be associated with variation in their natural composition and fermentation characteristics. Fruit substrates contain different concentrations of sugars, organic acids, water, and naturally occurring volatile compounds, which may influence the characteristics of the fermented bait. The fermentation process using baker's yeast may produce carbon dioxide, ethanol, and other fermentation associated compounds. Carbon dioxide is known to contribute to mosquito host seeking, while other odor cues can also influence mosquito orientation and attraction. However, because CO₂ production and volatile compounds were not measured in the present study, these factors should be considered possible explanations rather than confirmed mechanisms.

The sugar only control showed lower observed attraction than the fermented fruit-based treatments. Because the control did not contain fruit or yeast, its response cannot be directly interpreted as a comparison between fruit fermentation and sugar yeast fermentation. The plum-based bait (T6) also showed good attractancy, ranking second after guava. Plum contains high in alcohols and aldehydes profile, promotes vigorous fermentation and releases fruity esters which when fermented, can produce similar attractant VOCs. Banana bait (T12), although rich in ethene and fiber, was at third rank in attracting mosquitoes among the fruit-based baits. Remaining fruit-based baits also showed more attractiveness for the mosquitoes as compared to the control. The screening bioassay evaluated all twelve fermented fruit-based treatments in four groups of three treatments, with the sugar only control included in each cage. The treatment combinations were kept consistent for both mosquito species. Guava, plum, and banana showed comparatively higher observed attraction than the other tested fruit-based treatments and were therefore selected for further evaluation during the post screening phase.

The continued higher observed attraction of guava during the screening phase suggests that this fruit-based bait may have characteristics that are particularly attractive to the tested mosquitoes under the laboratory conditions used in this study. However, the specific chemical or fermentation related factors responsible for this response were not determined. Plum and banana also showed comparatively high observed attraction and were therefore retained along with guava for the post

screening bioassay. The selection of these three treatments was based on their observed performance during the screening phase rather than on direct measurement of CO₂ production or individual volatile compounds. The attractancy behavior of mosquitoes on guava bait was consistent and statistically significant ($p < 0.05$), indicating a strong preference. The continued high attractancy frequency suggests that guava fermentation generates a favorable and stable chemical profile that remains effective throughout the exposure period.

In contrast, although the plum bait attracted more mosquitoes than other ASBs and the control, its performance was less consistent. This variability could be due to differences in the chemical composition or fermentation rate of the plum extract, which may not sustain an optimal level of VOC release over time. The control bait remained the least attractive, again confirming that sugar only control, while useful, may not replicate the natural complexity and appeal of real fruit fermentation. The post screening bioassay provided a final simultaneous comparison of the three selected fermented fruit-based baits, namely guava, plum, and banana, together with the sugar only control. Guava maintained the highest observed mean attraction for both *Aedes aegypti* and *Culex quinquefasciatus*, followed by plum and banana. The sugar only control showed the lowest observed attraction.

The consistency of the observed ranking across the three experimental phases suggests that the selected fruit-based treatments differed in their attractiveness under the laboratory conditions of the study. However, the results should be interpreted in relation to the experimental design and the absence of direct measurements of CO₂ emission and volatile compounds. The higher response to guava may be related to its natural chemical composition and the products generated during fermentation. Fermentation of fruit substrates can produce CO₂, ethanol, organic acids, and other compounds that may influence mosquito behavior. In addition, naturally occurring fruit odors may contribute to the overall odor profile of the bait. Nevertheless, these potential mechanisms were not directly tested in the present study and therefore require further investigation. The observed differences between *Aedes aegypti* and *Culex quinquefasciatus* may reflect species specific differences in host seeking behavior and responses to chemical cues. The attraction values were generally higher for *Aedes aegypti* than for *Culex quinquefasciatus* in the present observations. However, direct statistical comparison between the two species would require an experimental design with appropriate independent replication.

These results confirm that guava fermentation is a consistently superior attractant, possibly due to its chemical profile, Balanced sugar–acid profile; produces ethylene and fermentation behavior. The high level of consistency observed across all three phases of the experiment strengthens the reliability of these findings and reduces the likelihood of chance-based bias. Species-wise, *Aedes aegypti* showed slightly higher overall attractancy activity compared to *Culex quinquefasciatus*, suggesting that *Aedes* may possess a heightened sensitivity to guava-related VOCs. This observation aligns with prior research showing that *Aedes* mosquitoes rely more heavily on visual and olfactory cues for host-seeking and oviposition behavior (Reisen, 2010). The superior performance of guava + yeast bait can be explained by the combined effect of CO₂ release and VOC production during fermentation. Mosquitoes use carbon dioxide as a long-range attractant, which activates their host-seeking response, while other volatiles (e.g., esters, alcohols, acids) act as short-range cues to guide them toward the source (Gillies, 1980).

Guava is known to contain high concentrations of balanced sugar–acid profiles; produces ethylene that can mimic host odors. This biochemical synergy likely explains the enhanced attractiveness of guava bait. The findings of this study may have practical relevance for the development of low cost mosquito monitoring tools. Locally available fruit substrates are relatively accessible and can be prepared using simple fermentation procedures. Among the tested treatments, guava showed the highest observed attraction under the laboratory conditions used in this study. However, laboratory attractiveness does not necessarily indicate field effectiveness. Environmental factors such as temperature, wind, humidity, competing odors, mosquito density, and availability of natural hosts may influence bait performance under field conditions. Therefore, field-based evaluation is required before these fermented fruit-based baits can be recommended for operational mosquito surveillance or control. Future studies should also investigate fermentation duration, substrate concentration, yeast concentration, CO₂ emission rates, and the volatile compounds produced by different fruit-based treatments. Such investigations could help determine the factors responsible for the observed differences in mosquito attraction.

Moreover, these baits can be easily adapted for use in attractive toxic sugar baits (ATSBs) by adding safe, targeted toxins to the bait. Such formulations have been shown to reduce adult mosquito populations without harming non-target organisms when used responsibly (Kline, 2007). This approach also aligns with integrated vector management (IVM) principles, encouraging the use of culturally acceptable, sustainable, and participatory tools for vector control.

Conclusion

The present study evaluated the comparative attractiveness of twelve fermented fruit-based baits to *Aedes aegypti* and *Culex quinquefasciatus* under controlled laboratory conditions. Among the tested treatments, guava showed the highest observed mean relative attraction during the prescreening, screening, and post screening bioassays, followed by plum and banana. The

sugar only control showed comparatively lower attraction throughout the experimental phases. The consistent response observed for guava, plum, and banana resulted in their selection for the post screening bioassay. Overall, the findings indicate that fermented fruit-based baits can produce different levels of mosquito attraction depending on the fruit substrate used. The observed attraction may be associated with differences in fruit composition and fermentation characteristics. Fermentation may produce carbon dioxide and other compounds that can contribute to mosquito attraction. However, CO₂ emission rates and individual volatile compounds were not directly measured in the present study, and therefore the specific mechanisms responsible for the observed differences cannot be confirmed. The study provides preliminary laboratory information on the attractiveness of locally available fermented fruit-based baits to *Aedes aegypti* and *Culex quinquefasciatus*. Further research should investigate fermentation conditions, CO₂ production, volatile compounds, independent experimental replication, and field performance before these baits are considered for practical mosquito surveillance or control applications.

Recommendations

Further studies are recommended to evaluate the most promising fermented fruit-based baits, particularly guava, plum, and banana, under both laboratory and field conditions against *Aedes aegypti* and *Culex quinquefasciatus*. Future experiments should include independent biological replication, larger mosquito populations, and repeated trials to improve the reliability and reproducibility of the results. The selected fruit-based baits should also be evaluated for their attractiveness over longer periods and under different environmental conditions, including variations in temperature and relative humidity. Field evaluation using mosquito traps is particularly important to determine whether the attraction observed under controlled insectary conditions can be maintained in natural environments. The practical suitability, stability, preparation requirements, and cost of the most promising formulations should also be considered before their potential use in mosquito surveillance and management programs.

Author Contributions

All authors contributed equally in the manuscript.

Competing Of Interest

The authors declare no competing interests.

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