



Impact of Wild Yeast Added to Culture Media on *Drosophila* Abundance in and Around a Banana Market, Darangiri, Assam, India

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ABSTRACT

In the Darangiri area of Goalpara district, Assam, a comprehensive two-year survey was conducted to assess the abundance of *Drosophila* species in response to the introduction of wild yeast named bakhar in local alcohol production by the Rabha and Boro communities. The study focused on six different culture media: Corn meal (A), *Musa balbisiana* (B), *Musa assamica* (C), mixed fruit (D), Jonga (E), and Jaggery (F). Environmental factors, including temperature (26°C during monsoon), relative humidity (71.23% during monsoon), rainfall (295.11 mm, monsoon), and day length (13.24 hours, June, monsoon), were recorded in the analysis. A total of 11,784 ± 10.16 *Drosophila* was collected and identified across the six-culture media. *Musa balbisiana* (Athiya colla), the indigenous banana, exhibited the highest *Drosophila* abundance at 3877 ± 3.98. Notably, the *Musa balbisiana* culture media attracted the highest number of *Drosophila melanogaster*, especially during the monsoon period. Female dominance in abundance was observed, particularly during the post-monsoon period. The study revealed variations in male-female breakdown between different culture media. Day time abundance exceeded night time in each season, with June recording the highest daytime abundance (1331) compared to September night time abundance (858). Diversity indices, including Dominance Index (0.333 for male & 0.346 for female, monsoon), Simpson Index (0.791 for male & 0.796 for female, post monsoon), Shannon-Weinner Diversity Index (1.669 for male & 1.674 female, post monsoon), Evenness Index (0.884 for male & 0.889 for female, post monsoon), Brillouin Index (1.655 for male & 1.664 for female, post monsoon), Mehinick's Richness Index (pre monsoon male for 0.169 & 0.199 winter male) and Margalef's Index (0.705 post monsoon male & 0.734 winter male), were analyzed across different culture media and seasons. Consistent species diversity, with six taxa in each category, was observed across seasons and genders. Monsoon showed higher species diversity according to the indices. Overall, the study provides a systematic understanding of *Drosophila* abundance, may be influenced by the wild yeast added to the culture media, environmental factors, and seasonal variations.

Keywords: Wild yeast, *Drosophila* abundance, *Drosophila* and Culture media, Banana market, Indigenous banana

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INTRODUCTION

Drosophila, a cold-blooded insect, demonstrates optimal survival between temperatures of 12-21°C (Hoffmann, 2010). Their nutritional needs play a crucial role in various biological functions, affecting abundance, stress resistance, life-history traits and reproduction (Burger et al., 2007, Sisodia and Singh, 2012, Vieillard et al., 2016, Rodrigues et al., 2015 and Kristensen et al., 2016). As saprotrophs, *Drosophila* relies on microbes for nutrition, establishing intricate interactions with fruits and microbes (Hamby and Becher, 2016). *Drosophila melanogaster*, a generalist, utilizes a variety of fruits and vegetables for both feeding and reproduction, with larval food composition impacting developmental stages and life

cycles (Chippindale et al., 1998, Fanson et al., 2009, Kristensen et al., 2011, Rodrigues et al., 2015; Reiset et al., 2016). Even the morphologically modified ovipositor of *Drosophila suzukii* enables oviposition in ripening fruit (Atallah et al., 2014). Larval nutrition influences resistance to heat, cold, starvation and desiccation, with sex-specific patterns (Andersen et al., 2010, Kristensen et al., 2016, Reddix et al., 2013 and Nazario-Yepiz et al., 2021). Females of *D. melanogaster*, for instance, are impacted by the protein to carbohydrate (P:C) ratio in various biological activities (Lee et al., 2008, Fanson et al., 2009 and Rodrigues et al., 2015). *Drosophila* has a historical association with fruits such as bananas, grapes, and plums in overripe or fermenting conditions. Mashed banana, often combined with *Saccharomyces cerevisiae*,

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has been integral to drosophila culture (Spencer, 1950; Dobzhansky and Sokoloff, 1951). Different substrates and nutrients influence the fitness of transferred flies (Trajkovic et al., 2017) and the broad climatic tolerance of drosophila contributes to its widespread distribution, given its large host range of fruits (Srinath and Shivanna, 2014, Enriquez and Colinet, 2017, Kirkpatrick et al., 2018). The population dynamics of drosophila are influenced by factors such as temperature, humidity, rainfall, light duration, fruiting plants, and seasons (Parsons and Bock, 1979, Sandhyarani, 2010). Seasonality has observable effects on morphology and distribution, especially in rich environments (Kirkpatrick et al., 2018, Throckmorton, 1975, da Cunha et al., 1955). Maintaining sufficient sugar levels in culture media is crucial due to *Drosophila*'s preference for sugar-enriched fruits like bananas (Stocker and Gallant, 2008). Northeast India, renowned for its diverse banana species, including *Musa assamica* and *Musa balbisiana*, faces challenges such as banana dumping in its largest market, Daranggiri (Debnath et al., 2019 and Baruah and Sahoo, 2018). The study introduces the use of the antioxidant-rich *M. balbisiana* in drosophila attraction, along with the impact of wild yeast ('Bakhar' or 'Surasi') from local practices on culture media. This exploration aims to understand the distribution and abundance in the context of the region's largest banana market, incorporating insights into seasonality and olfaction, focusing on how *Drosophila* senses the smell of its food.

MATERIALS & METHODS

Study Area

This pioneering study conducted in Daranggiri, the largest banana market in India (Fig. 1), is the first of its kind (Das, 2020). It is located at 134 kilometres west of Guwahati city, Daranggiri coordinates are 25.9861° N latitude and 90.8956° E longitude (Dutta, 2003; Ali, 2022). Renowned for being India's largest banana market, Daranggiri encompasses a sprawling area of 40,000 sq ft, hosting a centre for rotten banana disposal (Fig. 2).

The Wild Yeast "Surasi or Bakhar"

In the dynamics of fruit fly abundance, yeast assumes a crucial role, as noted by Pearl et al. (1926) and Hoang et al. (2015). Yeast serves the dual purpose of inducing fermentation and leavening, as outlined by Britannica (2023). Notably, local traditions in the study area involve the collection of specific plants from nearby forests for yeast preparation, as depicted in Fig. 3. These plants are crafted into small balls, serving as a source for alcohol production with a distinctive fermented aroma. Interestingly, the locals maintain a veil of secrecy around the identity of these collected plants, reserved for the creation of Surasi or Bakhar.

Preparation of Culture Media and Collection of Flies

The six different culture media were prepared as follows:

(A) Corn meal: Boiled 100 g of corn meal with 50 mL of water, allowed to cool, and mixed with 4 g of commercial yeast powder (CYP). Kept for 24 hours, incorporating 5% propionic acid in 1% sodium bicarbonate (V/V) as an additive, following the standard *Drosophila* culture procedure, serving as the Control.

(B) Malbhog Banana fruit medium (*Musa assamica*): Utilized 100 g of blended ripened malbhog banana with 50 mL of liquefied agar. Modified from the standard medium (Stocker and Gallant, 2008) by replacing CYP with 20 g of Bakhar. Wrapped with aluminum foil and left to stand for 24 hours.



Fig. 1: Location map of the study area.



Fig. 2: Banana Garbage site

(C) Athiya Banana fruit (*Musa balbisiana*): Similar to culture media B but used blended ripened athiya banana with 20 g of locally prepared yeast instead of CYP. Wrapped with aluminum foil and left to stand for 24 hours.

(D) Mix fruit (lemon, grapes, and apple): Blended 30 g each of ripened lemon, grapes, and 40 g of apple, mixed well in 50 mL of liquefied agar. Added 20 g of Bakhar and allowed to stand for 24 hours.

(E) Jonga: Prepared from 100 mL of fermented jonga, a locally made alcohol containing rice, water, Bakhar or Surasi, and green chili. Fermented for at least 5 nights, now available for use as culture media.

(F) Jaggery: Boiled 100 g of jaggery in 50 mL of water for 30 minutes in a 1-liter borosil beaker. Added 20 g of Bakhar and allowed to settle in a wrapped condition for 24 hours.

Tools and Techniques for Data Collection

In this study, *Drosophila* collection involved the use of plastic bottles and an insect net in and around the market, with nighttime observations aided by a torchlight (Fig. 4). Statistical analysis, conducted with the PAS V4 tool, focused on diversity indices such as Dominance, Simpson index, Shannon index, Evenness, Brillouin index, Menhinick index, and Margalef index.



Fig. 3: Wild yeast.

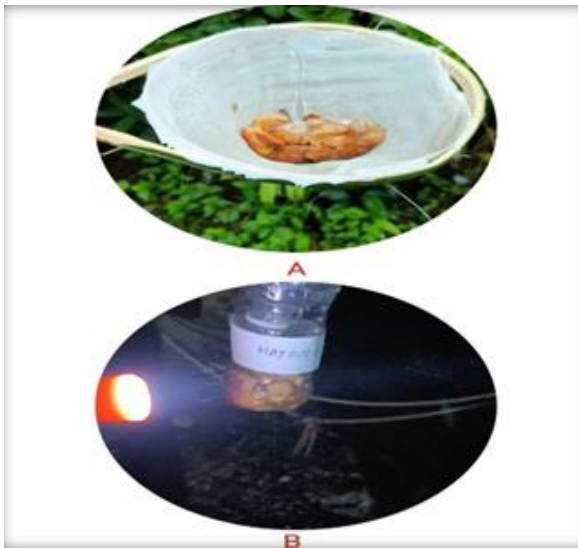


Fig. 4: Drosophila Collection method (A-Net sweeping & B- Traip bait)

Two collection methods were employed in tandem. The trap-bait method (Hegde et al., 2001) utilized 18 bottles, with three bottles for each culture medium (500 ml size plastic bottle) randomly tied to trees and left open for 24 hours. The net sweeping method involved spreading the culture media under a shaded area, sweeping performed after 24-30 hours, and precautions were taken to prevent rain entry into the bottles (Fig. 4). *Drosophila* collection occurred between January 2019 and December 2021, with day-night data recorded monthly during the study period (collection time: between 7 am to 1 pm and 5 pm to 11 pm). A total of 13 number of observations (Premonsoon 3, monsoon 4, post monsoon 3 and winter 3) were conducted. After collection, flies were etherized and transferred into the six mentioned culture media separately, placed in the laboratory, and observed under a microscope (Topal et al., 2021). The study period was categorized into four seasons: Pre-monsoon (March to May), Monsoon (June to August), Post-monsoon (September to November), and winter (December to February), following Borthakur (1986), with observations recorded for each season.

Record of Abiotic Parameters

Abiotic parameters such as Temperature, Relative Humidity (RH), and rainfall were meticulously recorded

using specific instruments. Temperature data were obtained using a maximum and minimum thermometer. The Relative Humidity (RH) readings were taken with the assistance of dry and wet bulb thermometers and an RH chart. Rainfall measurements were collected using a rain gauge.

Additionally, day length observations were conducted using a Sunshine recorder. These precise and varied methods ensured accurate recording of environmental factors, contributing to a comprehensive understanding of the study area's conditions during the research period.

Identification of Males and Females

Distinguishing between adult female and male flies involves a careful examination of their anatomy. In female flies, the abdomen serves as a prominent criterion for differentiation, as depicted in Fig. 5. Notably, adult female flies are considerably larger than their male counterparts. In males, the last two segments of the abdomen exhibit a darker shade compared to females. Specifically, males feature thick black bands on the abdomen, while females typically have one darker band at the bottom and a lighter band above it (Ashburner, 1989).

The shape of the abdomen is another distinguishing factor, with the female's abdomen being pointed, in contrast to the rounded bottom of the male's abdomen. Additionally, sex combs, characterized by thick black lines, are present on the forelegs of male flies, just before the joint, serving as a distinctive feature absent in females (Carrilo, 2022). These observable differences aid in the accurate identification of the sex of adult flies.

Statistical Analysis

In the exploration of variability and correlation among species and culture media, a diverse set of statistical parameters and diversity indices were systematically employed:

Statistical Parameters

1. Principal Component Analysis (PCA): Analyzed and represented variability among species and culture media.
2. Leading plot: Provided a visual representation of the leading factors influencing variability.
3. Bar chart: Illustrated the distribution and comparison of species and culture media.
4. Box plot: Displayed the distribution, variability, and outliers within different categories.
5. Radar chart: Offered a graphical representation of multivariate data, aiding in the comparison of species and culture media.
6. Euclidean cluster analysis: Applied to identify similarities among the six different culture media, facilitating a clustered representation.

Diversity Indices

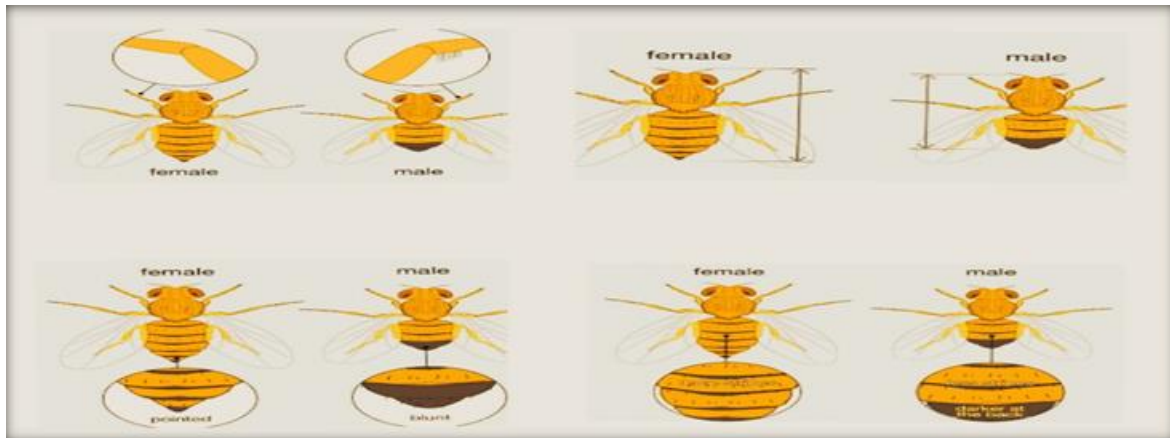
1. Taxa_S: Indicates species richness.
2. Individuals diversity: Measures diversity based on the number of individuals.
3. Dominance_D: Assesses the dominance of specific species.
4. Simpson_1-D: Reflects diversity, emphasizing dominant species.
5. Shannon_H: Quantifies diversity based on species abundance and evenness.
6. Evenness e^H/S : Represents the even distribution of species.
7. Brillouin: Measures diversity accounting for evenness and richness.

Table 1: Abiotic parameters of Goalpara district during the study Period.

Abiotic Factors ↓	SEASONS			
	Pre monsoon (March - May)	Monsoon (June - August)	Post monsoon (September - November)	Winter (December - February)
Temperature (°C)	22.42 ± 3.65	26.14 ± 3.5	21.22 ± 2.11	11.71 ± 1.27
RH (%)	51.75 ± 4.61	71.23 ± 5.65	71.65 ± 6.34	68.29 ± 5.24
Rainfall(mm)	93.39 ± 8.34	295.11 ± 12.39	207.57 ± 11.75	20.33 ± 3.34
Day length(h)	11.09 ± 0.63	13.24 ± 1.05	11.70 ± 1.01	10.84 ± 1.61

Table 2: Morphological characters of the recorded species.

Sr. No.	Species	Body colour	Eye Colour	Wing colour	Leg Colour
1	<i>Drosophila melanogaster</i>	Brown	Orange	Light brown	Light brown
2	<i>Drosophila pseudoobscura</i>	Blackish	Dark orange	Blackish	Brow-black
3	<i>Drosophila suzukii</i>	Light yellow	Black	Yellowish	Yellow-Black
4	<i>Drosophila immigrans</i>	Orange	Orange	Light yellow	Light yellow
5	<i>Drosophila sechelia</i>	Dark brown	Red	Yellowish	Yellowish
6	<i>Drosophila sophophora</i> (Sub genus)	Yellowish	Orange	Blackish	Yellowish

**Fig. 5:** Morphological differences of male and female drosophila.

- Menhinick: Assesses diversity considering species richness and sample size.
- Margalef: Reflects richness in proportion to the number of individuals.

This systematic approach ensures a comprehensive analysis, providing insights into the richness, dominance, and evenness of species in different contexts, as well as facilitating a nuanced understanding of variability, correlation, and similarities among species and culture media.

RESULTS

Temperature, RH, Rainfall and Day length of the study area has been depicted in Table 1. Six different drosophila species were identified as *D. melanogaster* (Dm), *D. pseudoobscura* (Dp), *D. suzukii* (Ds), *D. immigrans* (Di), *D. sechelia* (Ds1) and *D. sophophora* (Ds2) in six different culture media (Table 2). The abundance of species *Drosophila melanogaster* was maximum in each of the culture media compared to other drosophila species. The culture media with *Musa balbisiana* (local name-Athiya colla) attracted $6,732 \pm 3.79$ number of individuals, of which $3,876 \pm 3.95$ was in monsoon period. The lowest number was noted in the jaggery culture media at 453 ± 3.17 compared to other five media. The species richness found more in *Musa balbisiana* culture media (C) whereas it was less in Mix fruit culture media (Table 3). The total numbers of Drosophila were highest in Monsoon period followed by pre monsoon, post monsoon and winter (Table 3). Species Dm is present in all media, and other species (Ds1, Dp, Di, Ds2) are specific to certain media. Media F i.e. jaggery has the smallest difference between male and female counts. Culture media shows variations in both day

(D) and night (N) values across the months. Generally, day values are higher than night values (Table 4). The highest day value is observed in June, and the highest night value is in September. Day abundance records showed at its highest during June at 1331 ± 4.8 followed by July and August among the number of 11784 individuals recorded. However, the highest number of Drosophila was recorded in the night time of September 858 ± 2.51 compared to other months (Table 4) with an equal representation of male and female during the same period.

Statistical Result

The number of individuals varies across seasons and genders. Monsoon (females) has the highest number of individuals, followed by Pre-monsoon (females). Diversity indices analysis on the species diversity also tends to fluctuate with reference to different season and variation in the culture media (Table 5). Diversity analysis like Dominance_D revealed the highest in monsoon, 0.333 for male & 0.346 for female. Simpson_1-D show highest in post monsoon, 0.791 for male & 0.796 for female, Shannon_H diversity Index presents 1.669 for male and 1.674 female in post monsoon, Evenness Index represents 0.884 for male and 0.889 for female during post monsoon. Meanwhile Brillouin Index present 1.655 for male & 1.664 for female in post monsoon whereas Menhinick's richness Index represents 0.169 for male during pre-monsoon and 0.199 for male during winter season. Margalef's shows 0.705 for male in post monsoon and 0.734 for male in winter. Monsoon shows dominance for female with 0.3464 compared to others. Culture media with *Musa assamica* (B) found to be positively correlated with winter (males), Winter (female) in mixed fruit media found to be positively

Table 3: Abundance of *Drosophila* species and numbers against different types of culture media.

Culture Media	Sex	Number of <i>Drosophila</i> in Seasons (n= observations)				Number of Species	Total
		PM (n=3)	M (n=4)	POM(n=3)	W(n=3)		
A	(♂)	132±3.93(Dm)	160± 4.15(Dm)	65±1.68(Dm)	56±1.12, (Dm) 2.0±0.01(Ds1)	Dm=748±4.27 Ds1=6.0±0 .01	754±4.3
	(♀)	113±3.11(Dm)	153±4.07(Dm)	40±1.11(Dm)	29±1.03(Dm) 4±0.01(Ds1)		
B	(♂)	208±3.21(Dm) 2.0±0.1 (Dp)	392± 5.40(Dm) 4.0± 1.02 (Dp)	322±4.25(Dm)	223±3.62(Dm)	Dm=2593±7.09 Dp=7.0±0.61	2600±3.85
	(♀)	248±4.62(Dm)	498±7.01(Dm), 1.0±0.2(Dp)	356±4.55(Dm)	346±4.36(Dm)		
C	(♂)	297±4.01(Dm)	1221±9.25(Dm), 2±0.5(Di)	325 ±4.25(Dm)	281±3.86(Dm)	Dm=6729± 10.57, Di=2±.07 Ds=1±.01	6732±9.79
	(♀)	798±7.25(Dm)	2653±11.53(Dm), 1.0±.01(Ds)	465±8.87(Dm)	689±7.25(Dm)		
D	(♂)	47±6.33(Dm)	107±6.57(Dm)	95±7.21(Dm)	63±4.98(Dm)	Dm=761± 7.94	761±6.57
	(♀)	73±7.23(Dm)	216±7.39(Dm)	125±8.54(Dm)	35±5.76(Dm)		
E	(♂)	67±7.71(Dm)	113±8.22(Dm)	52±5.37(Dm)	61±3.11(Dm)	Dm=-482±5.98 Ds=2.0±0.7	484±5.84
	(♀)	42±4.31(Dm)	72±4.25(Dm)	46±3.35(Dm) 2.0±0.01(Ds)	29±2.29(Dm)		
F	(♂)	58±3.31(Dm)	63±2.89(Dm), 3..0±0.03(Ds2)	52±2.89(Dm)	13±1.54(Dm)	Dm=449±5.34 Ds2 =4±1.0	453±6.17
	(♀)	92±4.41(Dm)	71±3.15(Dm), ,1.0±0.01(Ds2)	68±3.59(Dm)	32±2.32(Dm)		

SEASONS: PM= Pre Monsoon (March to May), M= Monsoon (June to September), POM= Post Monsoon (October to November & W= winter (December to February): (♂)= Male and (♀) = Female: CULTURE MEDIA: A= Corn meal, B = *Musa assamica*, C= *Musa balbisiana*, D= Mixed fruit, E= Jonga & F= Jaggery: SPECIES: Dm= *D. melanogaster*, Dp= *D. pseudoobscura*, Ds= *D. Suzukii*, Di= *D. immigrans*, Ds1= *D. Sechelia* & Ds2= *D. sophophora*.

Table 4: Records of *Drosophila* available in day (D) and night (N).

Month	D/N	A	B	C	D	E	F	Total D/N
Jan	D	16±2.33	189±5.67	241±4.34	36±2.23	20±2.24	13±1.61	515±3.07
	N	12±2.21	87±5.31	35±3.20	13±2.11	06±1.31	03±1.22	156±2.56
Feb	D	26±3.12	102±7.64	289±8.23	15±2.34	24±2.84	11±1.83	467±4.33
	N	05±1.33	25±2.34	44±3.45	09±1.21	07±1.32	06±1.41	96±1.84
Mar	D	53±3.44	113±4.62	319±7.33	38±3.22	24±2.46	42±4.33	589±4.23
	N	21±2.33	46±3.56	134±8.45	08±1.21	08±2.21	20±1.21	237±3.16
Apr	D	63±3.21	104±5.22	287±7.22	25±3.44	37±3.23	22±2.33	538±4.10
	N	29±2.21	38±2.34	30±4.32	08± 2.11	04±1.01	13±1.12	122±2.18
May	D	57±3.33	147±6.12	269±9.21	33±3.42	23±2.12	36±3.12	565±4.55
	N	22±1.23	10±1.11	56±3.23	08±1.12	13±1.61	17±2.11	126±1.73
Jun	D	72±4.21	245±7.21	863±9.25	93±3.11	26±2.22	32±3.33	1331±4.88
	N	24±1.12	41±3.21	88±2.95	09±1.23	06±1.22	13±1.91	181±1.94
Jul	D	46±4.13	215±4.23	656±9.96	48±2.45	38±2.12	23±2.13	1026±4.17
	N	31±2.12	39±2.32	114±4.12	19±1.23	11±1.21	09±1.71	223±2.11
Aug	D	49±3.41	209±4.81	631±6.43	67±3.65	39±2.45	21±2.12	1016±3.81
	N	15±1.23	17±1.83	109±3.89	19±2.32	13±1.35	12±1.31	185±1.98
Sep	D	41±3.32	96±3.11	654±9.12	52±3.98	49±2.21	19±2.11	911±3.97
	N	35±2.88	33±2.22	762±5.42	16±2.23	03±1.21	09±1.11	858±2.51
Oct	D	46±3.45	268±5.23	367±3.67	151±4.12	46±3.42	59±3.12	937±3.83
	N	17±2.12	75±2.14	89±3.23	12±1.24	16±1.57	13±1.13	222±1.90
Nov	D	31±3.44	296±5.43	312±4.67	35±2.31	31±2.12	39±3.42	744±3.56
	N	11±1.23	39±2.34	22±2.11	22±2.21	07±1.21	09±1.23	110±1.72
Dec	D	17±3.12	129±4.12	296±4.55	18±1.95	21±2.11	07±1.21	488±2.84
	N	15±1.21	37±2.72	65±2.21	07±1.16	12±1.34	05±1.11	141±1.62
Total	D N	754±2.57	2600±3.95	6732±5.44	761±2.31	484±1.92	453±1.96	11784±10.16

Table 5: Diversity indices of *Drosophila* in different Culture media.

Diversity	Pre-monsoon (M)	Pre-monsoon (F)	Monsoon (M)	Monsoon (F)	Post-monsoon (M)	Post-monsoon (F)	Winter (M)	Winter (F)
Taxa_S	6	6	6	6	6	6	6	6
Individuals	1252	1711	2561	4585	1197	1685	909	1438
Dominance_D	0.225	0.219	0.333	0.346	0.208	0.203	0.260	0.263
Simpson_1-D	0.774	0.780	0.666	0.653	0.791	0.796	0.739	0.736
Shannon_H	1.641	1.658	1.417	1.381	1.669	1.674	1.544	1.529
Evenness_e^H/S	0.860	0.874	0.687	0.663	0.884	0.889	0.780	0.769
Brillouin	1.628	1.648	1.410	1.377	1.655	1.664	1.527	1.518
Menhinick	0.169	0.145	0.118	0.088	0.173	0.146	0.199	0.158
Margalef	0.701	0.671	0.637	0.593	0.705	0.673	0.734	0.687

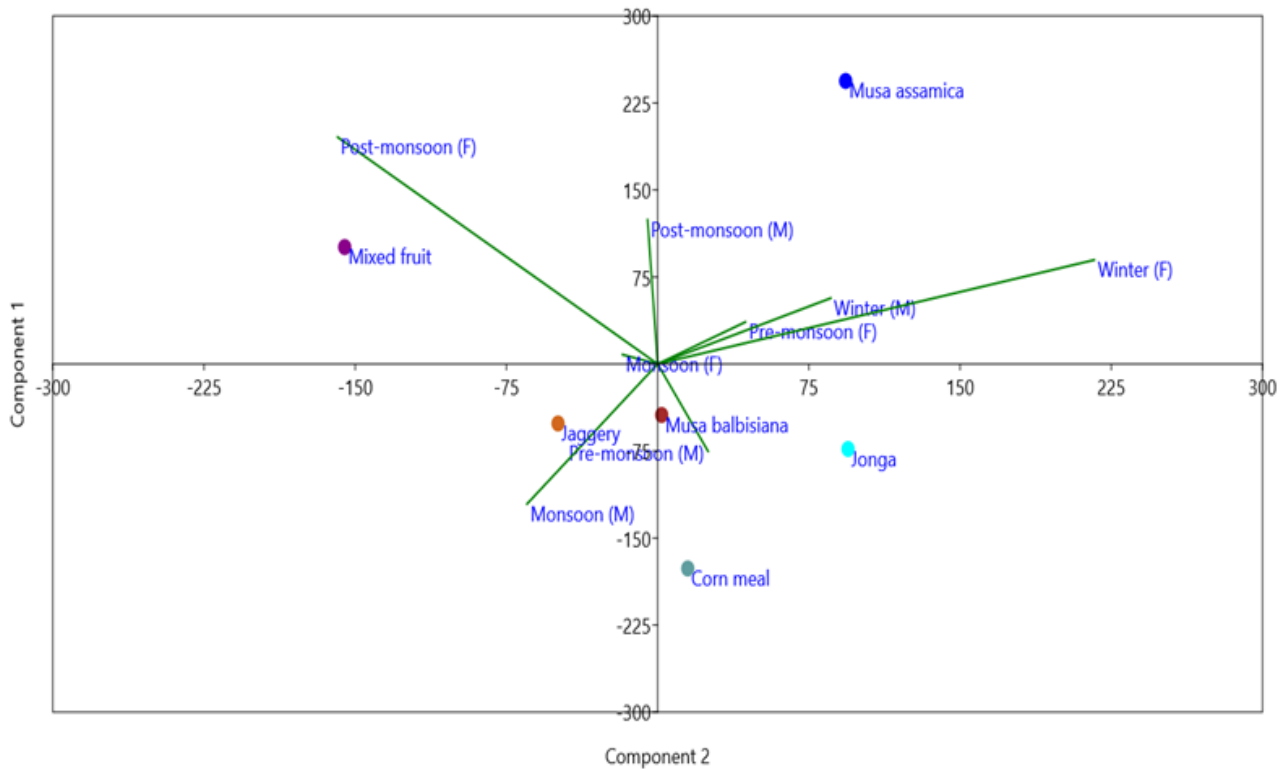


Fig. 6: PCA between the seasonality and various culture media in terms of drosophila abundance.

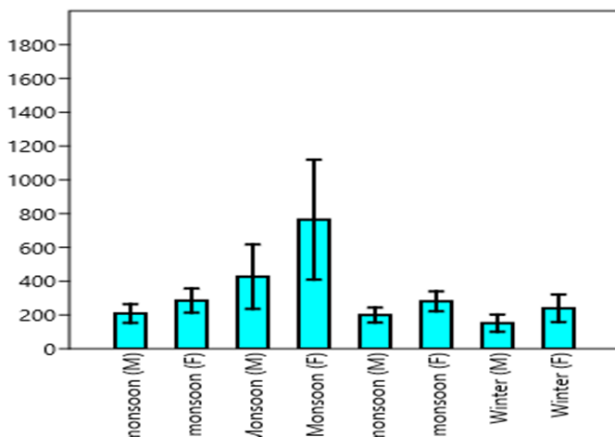


Fig. 7: Box Plot represents drosophila density against seasons.

correlated with the Post monsoon (female) and Post monsoon (male) as well as Monsoon (female), Jaggery was found to be positively correlated with Pre-monsoon (male) and Monsoon (male) in *Musa balbisiana* media. The corn meal and Jonga culture media has been recorded as the nonspecific one i.e. for all groups (Fig. 6). On the other hand, monsoon (female) *Drosophila* found to be at higher density or count during the study period (Fig.7). The radar Chart or DBA analysis presented impact of Monsoon (female) in all culture media (Fig. 8). The culture media *Musa balbisiana* represents the culture media similarity with other culture media as well as Jonga & Jaggery are also at close range with other media (Fig. 9).

DISCUSSION

The research conducted in Daranggiri, Asia's largest banana market, aimed to understand the abundance of

Drosophila species, particularly *D. melanogaster*, utilizing six distinct culture media. The region, often laden with rotten banana waste, provided a unique environment for the study. Various environmental factors, including temperature, rainfall, relative humidity and day length, were assessed for their impact on the abundance of *Drosophila*, with a specific focus on the monsoon season. The investigation revealed a notable preference for *D. melanogaster*, especially females, in the *M. balbisiana* culture media during the monsoon, as outlined in Table 3.

The investigation delved into the role of yeast in the fermentation of culture media. Utilizing the wild-type yeast known as "Bakhar or Surasi," the study highlighted its potential as an attractant for *D. melanogaster*. While the specific yeast species were not identified, the locally prepared yeast (Surasi or bakhar) emerged as an ideal component in the culture media. This yeast was suggested to act as an antifungal product and improve the pH of the culture media, impacting the concentration of nutritional compounds during the fermentation process (Becher et al., 2012, Lewis and Hamby 2019, Spitaler et al., 2020). The difference obtained in terms of species number and more numbers of *Drosophila melanogaster* might be related with the 'wild yeast used', which affected on the concentration of nutritional compound in the fermentation process (Spitaler et al., 2020). However, the present investigation has been failed to identify the type of yeast species, since the locals traditionally allow none to enter in this field.

However, Hoang et al. (2015) attempted to project that there was no preference for *Saccharomyces cerevisiae* over the naturally obtained or wild varieties. Since yeast interactions are more complex than those of the experimental status. Yet, this group of workers advocated towards naturally associated yeast. Perhaps the flies *D. melanogaster* respond to airborne volatile compounds as a part of attraction to the culture media (Hoang et al., 2015). The feeding behaviour data show that *D. melanogaster* prefers three yeasts (*H. occidentalis*, *H. uvarum*, and *B. naardenensis*) and shows no preference

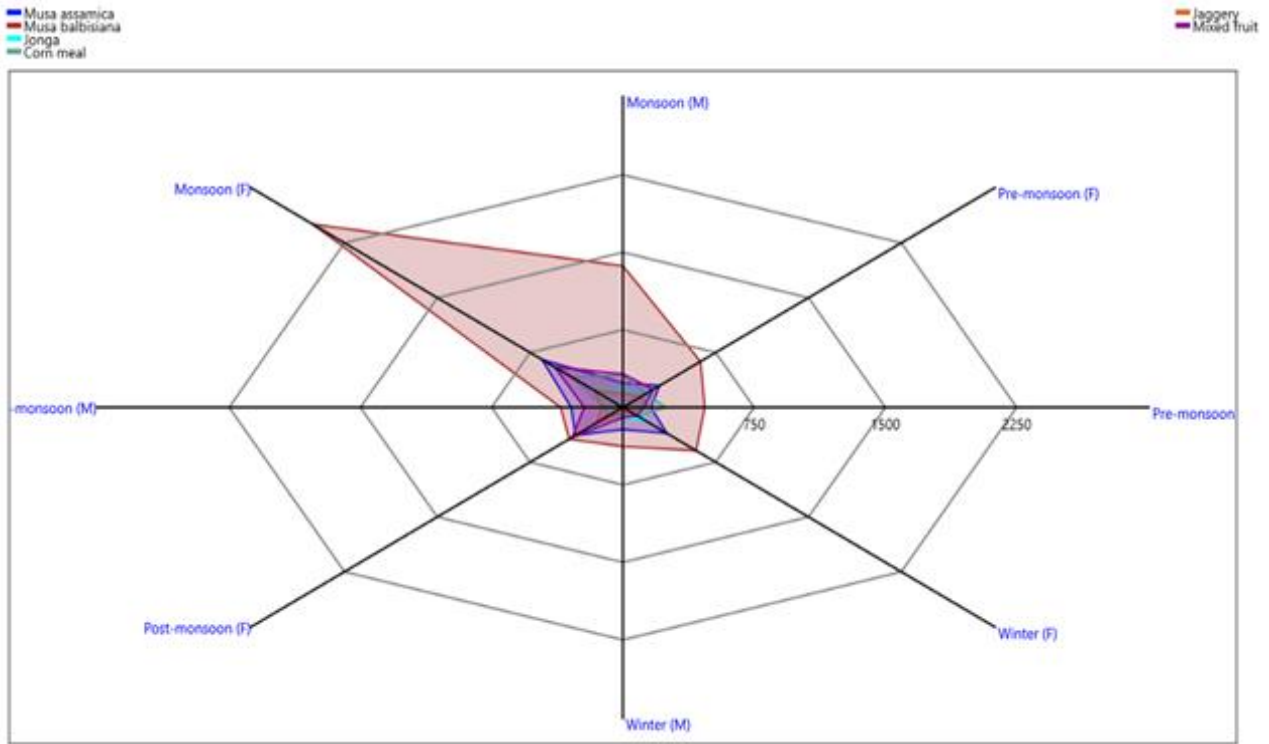


Fig. 8: Radar Chart (DBA) represents impact of season on the sexuality of drosophila in various culture media.

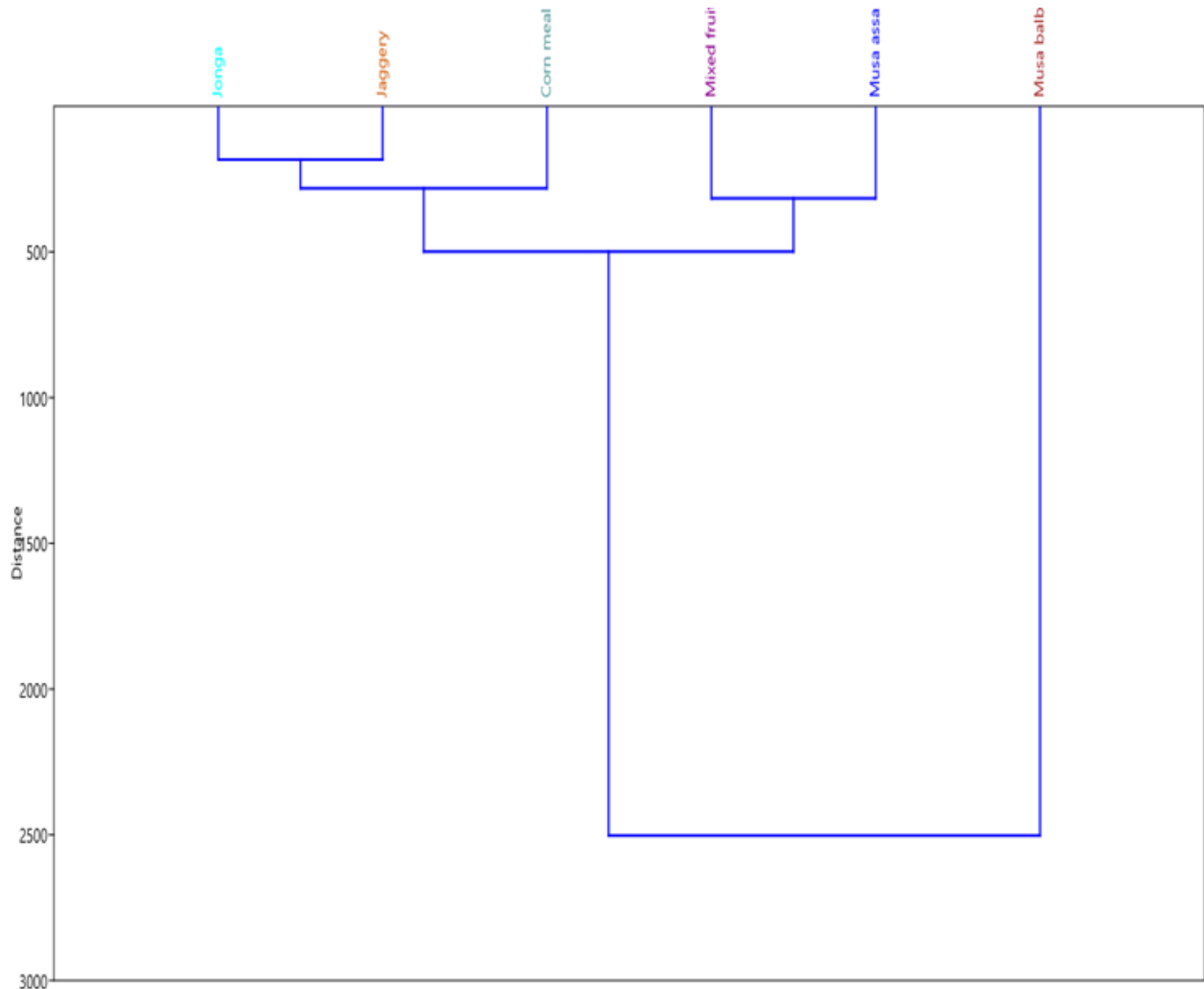


Fig. 9: Euclidian Cluster analysis for the effect of different culture media for similarity index.

for one yeast (*D. hansenii*). In one case (*S. paradoxus*), the naturally associated yeast was preferred when labelled blue, but not when labelled red. Overall, these studies suggest a general positive preference/attractiveness towards naturally associated yeast strains (Hoang et al., 2015). Therefore, it is encouraged that future would give emphasis on the yeast naturally associated with drosophila.

Monsoon period attracts higher numbers of drosophila compared to other seasons (Guruprasad et al., 2011). Abundance of *Drosophila* was more in day time than in the night time. Comparative study concerning the variation in season and culture media in this region of Assam, India will enable us to understand the concept of seasonality and olfaction to sense the smell of their food. Further, the pattern of distribution of species in any ecosystem depends on various factors like geographical, environmental, biological or abiotic (McCoy, 1990, Parsons and Bock 1979, Sanders, 2022). The culture media with *Musa balbisiana* (C) as the most suitable culture media may be due to its nutritional richness compared to other five media (Kalita et al., 2015, Sarma et al., 2022) which might be one of the reasons of *Drosophila* abundance. Interestingly the *D. melanogaster* was present in all culture media, suggesting a general adaptability or preference for the conditions provided by these media. Seasonal variability, particularly during the monsoon, played a crucial role in *Drosophila* abundance and diversity. Diversity indices highlighted the impact of seasonality, with monsoon recording the highest dominance, especially among female *Drosophila* (Guruprasad et al., 2010, 2011).

Drosophila melanogaster against a very little presence of *Drosophila suzukii* (Table 3) in the culture media added with *Musa balbisiana* (C). The effect of rainfall has been shown to be profound compared to temperature (Dobzhansky and Pavan, 1950), which influences on the increase of numbers even during the study period. It was also observed that the abundance of species *Drosophila melanogaster* contains i.e. $11,762 \pm 6.19$ individuals out of total $11,784 \pm 10.16$ (Table 3). Month wise analysis on the abundance of drosophila presented the September (Table 4) as the ideal month since both day and night period were with identical numbers of individuals of *Drosophila melanogaster*. The day time also shows that drosophila individuals were mostly attracted during the period of Monsoon (Table 4). Light (day time) and dark (night time) regime usually mimics seasonality causing behavioral adjustments while altering clock gene expression (Majercak et al., 1999, Shafer et al., 2004). *Drosophila* under a wide range of photoperiod condition presented the timing of the overt rhythm has been systematically changing (Pittendrigh et al., 1981), however such presentation was not possible in this study.

The effect of monthly temperature on total abundance was driven largely by the highly abundant *D. melanogaster* (Molon et al., 2020). The average amount of precipitation per month also influences total abundance and that of the most represented species of drosophila, with the exception of *D. affinis*. The lack of a significant monthly precipitation effect on the *D. affinis* that collected is especially interesting as *D. affinis* consisted of a substantial proportion of all collections (Bombin and Reed, 2016), meaning that the other species were driving the variation in total abundance in response to monthly fluctuation. We can conclude that difference in climate variables per season produces a more significant effect on the abundance of drosophila than temperature and precipitation during collection days. In addition, we can see that most *Drosophila* species exhibit a quadratic response to a seasonal climate variation, suggesting the presence of an optimal climatic condition range for each species.

Several previous studies reported no significant correlation between abundance of *Drosophila* and seasonal temperature variation (Guruprasad et al., 2010, Srinath and Shivanna, 2014, Torres and Madi Ravazzi, 2006). The reason for the difference in our results relative to theirs could be due, in part, to the different approach in statistical analyses. Most of these studies used a linear regression model. However, living organisms have an optimal range of climate conditions to which *Drosophila* exhibits different temperature tolerance (Hoffmann, 2010, Kellermann et al., 2012). In addition, Poppe et al. (2015) showed negative correlation between drosophila abundance and maximum/ minimum temperatures, which further suggests that drosophila are mostly abundant in a temperature range between the extreme values. The quadratic model appears to be the most appropriate for analysing the influence of temperature on the abundance of drosophila species and allowed to identify optimal condition ranges. For the most abundant species, their optimal monthly average temperature ranged from 18 to 26°C also supports the present findings. In addition, ecological data are often over-dispersed (Kindt and Coe, 2005), and several studies indicate that a negative binomial model, as used and the quasi-Poisson model are more appropriate in analyses of such data.

The investigation identified the monsoon period as particularly attractive for *Drosophila*, with higher abundance during the day compared to the night. The comparative study in Assam aimed to elucidate seasonality and olfaction's role in sensing food odors. Factors influencing species distribution in ecosystems, such as geographical, environmental, biological, or abiotic factors, were acknowledged. The nutritional richness of *M. balbisiana* (C) culture media was proposed as a contributing factor to increased drosophila abundance. Notably, *D. melanogaster* demonstrated adaptability to all culture media, suggesting a general preference for the conditions provided. Findings from Dharwad district, India, supported the notion of higher *D. melanogaster* numbers during the monsoon season, attributing population fluctuations to environmental variations (Table 3).

The investigation touched upon the role of sexual dimorphism and pheromones, particularly cis-vaccenyl acetate (cVA), in influencing *D. melanogaster* attraction to food odours. The study highlighted the interconnectedness of pheromone and food odour communication, with female-produced sex pheromones playing a crucial role in modulating attraction based on the nutritional state (Lebreton et al., 2015). A sexually dimorphic behavioural response to cVA, i.e. increased female receptivity to male courtship vs. male-male aggression and courtship inhibition, relies on sexually dimorphic third-order neurons (Zhou et al., 2014, Cachero et al., 2010, Datta et al., 2008, Ruta et al., 2010, Kohl et al., 2013). Fruit flies *Drosophila melanogaster* gather and mate on decaying and fermenting fruit. Yeast growing on fruit serves as an essential part of the adult and larval diet and flies are accordingly attracted to fermentation metabolites. During mating, males release the volatile sex pheromone cis-vaccenyl acetate (cVA), which increases female receptivity (Lebreton et al., 2015) and functions as an aggregation pheromone, since it enhances male and female attraction to food odour (Kurtovic et al., 2007 and Bartelt et al., 1985). Odours emanating from food also act as aphrodisiacs by themselves and promote male courtship (Lebreton et al., 2012 and Grosjean et al., 2011) which further emphasizes the interconnection between pheromone and food odour communication in female flies modulates cVA perception in first-order olfactory neurons. Starvation increases, and feeding reduces attraction to

food odour, in both sexes. Adding cVA to food odour, however, maintains attraction in fed females, while it has no effect in males (Lebreton et al., 2015).

Impact of season on various category of diversity could be understood from the diversity indices (Table 5) in terms of seasonality. However, the *Drosophila* diversity is least influenced simply by species richness. (Torres and Madi-Ravazzi 2006 and Achumi et al., 2013). The dominance recorded for the total taxa of six at 0.3464 in the monsoon. Interestingly the female *Drosophila* out of these taxa was higher in the monsoon period (Guruprasad et al., 2010). The present findings, even represented through the Box plot analysis for female *D. melanogaster* during monsoon (Fig. 7) could also be supported by Radar chart analysis (Fig. 8). Euclidian cluster analysis (Fig. 9) for similarity index revealed two major cluster banana mash, supports that *M. balbisiana* could attract many more flies. Seasonality on the abundance and diversity of *Drosophila* are better explained by temperature variation (da Mata et al., 2015), shared that *Drosophila* are sensitive to temperature and precipitation (Bombinand Reed, 2016). The present study has put forwarded the highest numbers of fruit fly attracted by the culture media (C) i.e. with *M. balbisiana* (Table 3) followed by the culture (B) i.e. *M. assamica*. However, it is not understood how these two-culture media with two indigenous banana mash had been able to attract females, perhaps due to the presence of antioxidant (Debnath et al., 2019 and Sarma et al., 2022).

Principal Component Analysis (PCA) suggested that banana mash (*M. balbisiana*) was more effective in attracting *D. melanogaster*, attributed to its rich nutrient content (Sarma et al., 2022). The study further explored the influence of climate variables, noting that monthly temperature and precipitation significantly affected *Drosophila* abundance. The quadratic model revealed optimal monthly average temperature ranges for the most abundant species (Dobzhansky and Pavan, 1950; Hoffmann, 2010; Kellermann et al., 2012).

The investigation emphasized the potential avenues for future research to address remaining questions and inconsistencies. Specifically, it called for a more in-depth exploration of naturally associated yeast strains and their intricate interactions with *Drosophila*. Additionally, the study suggested further exploration into the mechanisms behind the attractiveness of *M. balbisiana* banana mash, considering its rich nutrient content. Future research endeavors could focus on refining statistical analyses, considering the quadratic model's appropriateness, and exploring optimal climatic condition ranges for different *Drosophila* species. Overall, the study provides a comprehensive foundation for future research directions in understanding *Drosophila* ecology, yeast interactions, and the impact of environmental factors on population dynamics.

Conclusion

The current study delved into the species abundance of *Drosophila* in and around the largest banana market, a site with garbage dumping, marking the first-time inclusion of such an environment in biodiversity surveys. An attempt was made to demonstrate that endemic *Drosophila* species exhibit sensitivity to climatic variables (Parsons, 1991), revealing a significant correlation between the most abundant endemic species and seasonal shifts. Seasonal temperature, rainfall, relative humidity (RH), and day length variations collectively influenced the overall abundance of *Drosophila*. Intriguingly, the invasive species *D. suzukii* exhibited the least abundance and demonstrated no significant correlation with overall abundance and seasonality (Ferreira and Tidon, 2005).

Day length also potentially impacted the abundance of *Drosophila melanogaster*, with September emerging as an ideal month.

The study proposed that culture media containing *M. balbisiana*, followed by *M. assamica* in the presence of wild yeast, could be a valuable resource for *D. melanogaster*. Despite the garbage being comprised of rotten bananas, the study could not establish the diversity of the *Drosophila* group comprehensively. Additionally, it revealed a higher attraction of females compared to their male counterparts. However, the study acknowledged the existence of many unresolved questions that require in-depth exploration.

Future research avenues could involve a detailed investigation into the factors contributing to the sensitivity of endemic *Drosophila* species to climatic variables. Furthermore, understanding the reasons behind the lower abundance of invasive species like *D. suzukii*, despite the presence of favorable conditions, could provide valuable insights. Exploring the intricacies of attraction mechanisms, particularly focusing on the higher female attraction, may unravel novel aspects of *Drosophila* behavior. Additionally, a more thorough examination of the dumped rotten banana garbage's impact on the overall diversity of the *Drosophila* group would contribute to a more comprehensive understanding of the ecosystem. These avenues for future research aim to build upon the present study, offering a more nuanced and detailed perspective on *Drosophila* ecology in environments characterized by garbage. This study holds paramount importance for several reasons. Firstly, understanding the sensitivity of endemic *Drosophila* species to climatic variables is crucial for predicting and mitigating the impact of climate change on biodiversity. By elucidating the factors that influence their abundance, conservation efforts can be more precisely tailored to protect these species in the face of changing environmental conditions. The enigma surrounding the lower abundance of invasive species like *D. suzukii*, despite seemingly favorable conditions, underscores the need to explore the intricacies of ecological interactions. Unraveling the factors that limit the success of invasive species can inform strategies for managing and preventing their proliferation, which is essential for preserving the balance and integrity of local ecosystems.

The study's revelation of a higher attraction of females compared to males raises intriguing questions about the underlying mechanisms and evolutionary implications. A detailed investigation into the reasons behind this sexual dimorphism in attraction can contribute not only to our understanding of *Drosophila* behavior but also to broader insights into mating strategies and reproductive dynamics in insect populations. Furthermore, the study's inability to establish the diversity of the *Drosophila* group in a garbage-dumping environment highlights the gaps in our knowledge regarding the impact of anthropogenic waste on insect communities. Addressing this knowledge gap is imperative for developing effective waste management strategies that consider and mitigate potential ecological consequences.

In essence, tackling the unresolved questions is not merely an academic pursuit; it is a critical step towards advancing our understanding of the intricate relationships between *Drosophila* species and their environment. This knowledge, in turn, has practical implications for conservation, invasive species management, and sustainable waste disposal practices, contributing to the broader goals of ecological preservation and biodiversity conservation.

Conflict of Interests

The authors declare that there is no conflict of interest.

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