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Seasonal prevalence of blow Flies (Diptera: Calliphoridae) and bacterial load on their body surface in Hihya region, Al-Sharqia Governorate, Egypt

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Abstract

Calliphoridae (Blowflies) are a dangerous group of mechanical vectors of many microbes. The present work was carried out to study the prevalence and diversity of adult Calliphoridae flies and to isolate and identify bacterial strains from their external surface. A total of 558 Calliphoridae adult flies belonging to subfamily (Chrysomyinae, Luciliinae), two genera (Chrysomya, Lucilia) and six species (*Lucilia sericata*, *Chrysomya albiceps*, *Ch. marginalis*, *Ch. putoria*, *Ch. megacephala*, *Ch*. *Bezziana*) were collected from Hihya in middle Al-Sharqia Governorate, Egypt through the study time from April 2023 to March 2024. The abundance and diversity of subfamily Chrysomyinae (418 individual, 74.91%) were more than subfamily Luciliinae (140 individual, 25.09%). Temperature and humidity had a major role in the density of flies during the study period. The type of bait (fish or liver or meat) to attract adult insects had an impact on their density and diversity. There was also evidence of a single-year variance regarding files distribution and abundance of bacterial isolates between seasons, which could be attributed to weather. After isolation and identification fifth species were discovered as following, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*. The incidence of the microbial species did not show any significant change throughout different seasons however, it was appeared to be significantly varied according to the type of flies.

Keywords: Calliphoridae, Prevalence, Abundance, Seasons, Bait Traps, Pathogenic bacteria

1. Introduction

Flies are divided into three groups: flies of criminal importance, flies that transmit diseases, and flies that cause myiasis (Alikhan *et al.* 2018). The main flies that mechanically transmit a variety of diseases, including cholera, enterovirus, trachoma virus, bacillary dysentery, and tuberculosis, poliomyelitis and typhoid belong to the families Muscidae, Sarcophagidae, and Calliphoridae (Grassberger *et al.* 2003; Hanan 2013). Calliphoridae (blow flies) are usually associated with rotting materials. Most species of larvae are found feeding on the decaying remains of vertebrate animals, while some other species were found feeding on living tissue or can survive on other alternate sources such as feces. Because these insects usually feed on rotting materials, they are closely related to microbes, and therefore have great medical and veterinary importance in cases of infections, such as bacteria, viruses, protozoa, and parasitic worm, among humans and other animal species (Tomberlin *et al.* 2017 and Dar and Mir 2024).

Non-biting flies are found in residential and agricultural areas, as they are closely related to human and animal life (Yu *et al.* 2018). Non-biting flies often contaminate food, animal feed, and meat through contact with the body surface (Ahmadu *et al.* 2016). Globally, Calliphoridae (blow flies) have been recorded as vectors with the highest prevalence of parasites among non-biting flies (Liu *et al.* 2023). It has been demonstrated that dipteran flies spread germs through their spongy mouthparts, vomiting, body and leg hairs, sticky areas of the feet, and the digestive tract, contaminating food and spreading illness (De Jesús *et al.* 2004). Adult flies stay in microbe-rich environments, like those contaminated by animal waste, to feed on decomposing materials, such as manure and animal fluids, as well as ruined food and beverages (Neupane *et al.* 2019; Yin *et al.* 2022).

Furthermore, research on the microbiome of dipteran flies contributes to our understanding of the pathogenic agents that house flies interact with and how they spread disease (EL-Ghwas *et al.* 2021). Studies have revealed a wide diversity of bacteria isolated from the surface of insects, including both gram-positive and gram-negative species (Junqueira *et al*. 2017). Some of the commonly isolated bacterial genera include Bacillus, Pseudomonas, Staphylococcus, Enterobacter, and Serratia (Prabhukarthikeyan *et al*. 2014). These bacteria may have various roles in insect biology, such as nutrient provisioning, protection against pathogens, and communication (Engel & Moran, 2013). In addition to the role of flies in transmitting pathogens that infect humans and animals, they have an economic impact on animal production because of their infection of livestock with myiasis. This leads to reduced reproductive capacity and productivity, decreased milk production, blindness, muscle injury, lameness, and mortality of offspring in farm animals (Sotiraki and Hall, 2012). Although myiasis has been known since ancient times as a major disease (Sherman 2000), in recent years the disease remains poorly controlled in many regions of the world, this results in the death of animals and significant financial losses (Sotiraki and Hall, 2012).

The present study was carried out to determine the prevalence and diversity of adult Calliphoridae flies in Hihya area, Sharkia Governorate, Egypt, and to isolate and identify bacterial strains from their external surface. This study is the first of its kind in Al-Sharqia Governorate, Egypt, and represents a preliminary evaluation to determine the role of blow fly species in the mechanical transmission of pathogenic bacteria.

2. Materials and Methods

2.1 Survey of Calliphoridae flies

2.1.1 Study sites

The survey of flies causing myiasis was conducted in agricultural area of Al-Sharqia Governorate, Egypt namely: Hihya (30°39’ 43.0"N, 31°34'59.6"E). Hihya is a city located in the Sharkia Governorate, which is in the northeastern part of Egypt: Hihya is situated in the eastern part of the Nile Delta, approximately 80 kilometers (50 miles) northeast of Cairo, the capital of Egypt (Figure 1). Agriculture plays a significant role in Hihya 's economy, with the fertile Nile Delta soil supporting the cultivation of crops such as cotton, wheat, corn, and vegetables. Hihya experiences a hot desert climate, with hot, dry summers and mild winters. The city receives very little rainfall throughout the year.

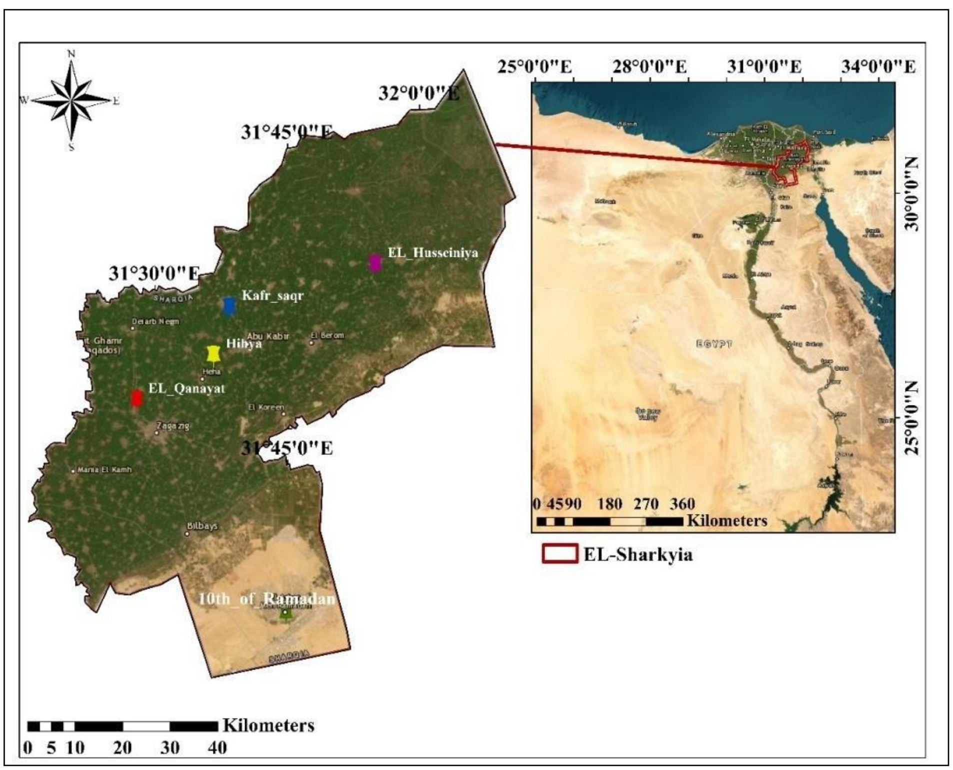

Figure 1. Study site.

2.1.2 Insects collection and sampling

To collect the adult flies, standard baited tarps of Lindsay *et al.* (2012) were used with some modification. Agricultural areas were chosen in Al-Sharqia Governorate region. Three types of baited trap (meat, liver, and fish) were used. Survey period extended from April 2023 to March 2024, the study site was visited once a month to collect adult flies. Trap was 28 × 20 cm dimensions and 13 cm height with plastic cover contains 8 holes, the entrance holes had conical collars inserted in the trap each 1 cm in length and 0.6 cm in diameter at the smallest opening and pointing into the trap chamber. Inside trap 50 g of bait was put down (Figure 2). The traps were suspended about one meter above the ground. Flies were collected after two days. Samples were examined and separated from the baits and transferred to the laboratory to complete the separation and identification processes. After collection, adult flies were preserved in vials labeled with date and time of collection. Collected insects were identified to species by using different keys (Sabrosky 1951; Shaumar *et al.* 1989; Setyaningrum and Al Dhafer, 2014).

Figure 2. Bait tarps showing (**A**) Liver bait, (**B**) Meat bait, and **(C**) Fish bait.

2.2 Climatic conditions

The main weather factors in the present study (max. & min. temperature, relative humidity, and wind Speed) were obtained from the Egyptian Meteorological Authority is the governmental organization.

2.3 Isolation and identification of associated bacteria

Different types of enrichment and selective media were used for isolation and identification of bacteria Nutrient Agar medium, MacConkey agar, Mannitol salt Agar, Rappaport-Vassiladis (RVS) enrichment broth media, XLD Test. Cetrimide Agar, Eosin Methylene Blue (EMB) Agar, MacConkey Broth (Purple). There are some other tests that have been done to identify organisms, such as Gram's reaction, Oxidase test, and Slide and tube coagulase test (Said, *et al*. 2021 and Zaher, *et al*. 2023). Pure colonies were examined for the biochemical identification of bacteria via Vitek 2 system according to the supplier instructions**.**

2.4 Statistical analysis

The statistical analysis was performed using GraphPad Prism 10.2.2. Using the analysis of variance either one way of two way (ANOVA), the correlation matrix between the different correlated parameter was also, done. The post hock analysis was performed with Tukey test. The values indicated significant difference when $p<0.05$ regarding ANOVA test. Meanwhile, the negative values for the correlation coefficient factor indicate reverse relationship and vice versa.

3. Results

3.1 Diversity, abundance and seasonality of Calliphoridae flies

The relative abundance and diversity of Calliphoridae flies are given in Table 1. A total of 558 fly Calliphoridae adults belonging to two genera (*Chrysomya*, *Lucilia*), two subfamilies (Chrysomyinae and Luciliinae) and six species were collected during the study period. The genus *Chrysomya* was more diverse with five species, followed by *Lucilia* with one species. Chrysomyinae insects were most abundant with the highest percentage (74.91 %) of total collected insects. This Subfamily was represented by 5 species namely, *Ch. albiceps*, *Ch. bezziana*, *Ch. putoria*, *Ch. marginalis* and *C. megacephala*. The highest density of this Subfamily was *Ch. megacephala* with 42.83% of total collected insects. Meanwhile, *Ch. bezziana* and *Ch. putoria* were represented by the lowest value (3.05 and 2.15 %, respectively). The density of Luciliinae was 140 individuals representing 25.09% of the total insects collected. The relative abundance, diversity and seasonality of subfamily Chrysomyinae and Luciliinae were more in the summer season (July, August, and September) with 9.3%, 13.4%, 10.6% for Chrysomyinae and 3.2%, 4.8%, 3.6%, for Luciliinae. Table 2 shows that the total number of flies collected was highest in the summer with a rate of 44.9%, followed by the spring with a rate of 24.6%, then decreased to 22.6% in the autumn, and the numbers of species were lowest in the winter with a rate of 7.9%.

3.2 Effect of temperature, relative humidity, wind speed on abundance and seasonal diversity of Calliphoridae flies

Monthly climate conditions from April 2023 to March 2024 in the city of (Hiya), Al-Sharqia Governorate was recorded in Table 3. The highest mean temperature (31.49, 31.07 and 30.03 °C) was recorded in summer season

(July, August and September) and while the lowest mean temperature (14.39, 14.67 and 18.04 ˚C) was recorded in winter season (Jan., Feb., and March). The relative humidity (RH) was moderate in summer season (July, August and September) where it recorded (45.4, 47.4 and 49.12%), respectively. Also, the wind speed recorded was moderate in summer, recording averages (2.73, 2.68 and 2.41 m s-1), respectively. The correlation matrix between flies incidence and different environmental factors illustrated in Figure 3, the occurrence of the all isolated species displayed a strong direct correlation with temperature, mild direct correlation with the wind speed, and strong inverse correlation with relative humidity.

Table 1. Monthly abundance and diversity of flies collected by baited traps from the Agricultural area (Hihya), Middle Al-Sharqia Governorate during the period from April. 2023 to Mar. 2024.

Sub Family	Species	The monthly abundance of flies collected												Total No.	%
		Apr.	May	Jun.	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.		
Chrysomyinae	*Chrysomya albiceps*	3	9	0	9	14	8	6	5	4	2	2	2	64	11.47
	Chrysomya bezziana	0	3	0	6	0	5	2	0	1	0	0	0	17	3.05
	Chrysomya putoria	2	0	0	3	4	0	1	0	2	0	0	0	12	2.15
	Chrysomya marginalis	12	7	6	10	13	7	10	7	7	3	2	2	86	15.41
	Chrysomya megacephala	25	17	14	24	44	39	21	19	15	10	6	5	239	42.83
Total (%)		42 (7.5)	36 (6.5)	20 (3.6)	52 (9.3)	75 (13.4)	59 (10.6)	40 (7.2)	31 (5.6)	29 (5.2)	15 (2.7)	10 (1.8)	9 (1.6)	418	74.91
Luciliinae	*Lucilia sericata* (%)	10 (1.8)	15 (2.7)	14 (2.5)	18 (3.2)	27 (4.8)	20 (3.6)	12 (2.2)	8 (1.4)	6 (1.1)	6 (1.1)	2 (0.36)	2 (0.36)	140	25.09
Total No. of Subfamilies (%)		**52 (9.32)**	**51 (9.14)**	**34 (6.09)**	**70 (12.55)**	**102 (18.28)**	**79 (14.16)**	**52 (9.32)**	**39 (6.99)**	**35 (6.27)**	**21 (3.76)**	**12 (2.15)**	**11 (1.97)**	**558**	

Table 2. Total number and percentage of Calliphoridae files collected during different seasons

No.	Season	Total No. of flies collected	%
1	Spring	137	24.6
2	Summer	251	44.9
3	Autumn	126	22.6
4	Winter	44	7.9

Table 3. Monthly fluctuation in climatic conditions from April 2023 to March 2024 in (Hihya), Al-Sharqia Governorate

Month	Temperature ºC			R.H (%)	W. Speed (m s-1)
	Max.	Min.	Mean		
April 2023	31.00±3.92	13.76±2.37	21.70±2.79	47.11±8.80	2.73±0.91
May 2023	34.35±2.88	17.43±2.34	25.36±2.28	46.85±7.23	3.17±0.92
June 2023	38.47±2.19	21.27±1.86	29.37±1.85	42.80±6.70	3.10±0.69
July 2023	41.08±1.82	23.25±1.24	31.49±1.48	45.40±6.23	2.73±0.55
August 2023	40.51±1.43	23.45±0.99	31.07±1.13	47.40±6.94	2.68±0.48
September 2023	38.81±1.92	22.75±1.88	30.03±1.69	49.12±7.89	2.41±0.56
October 2023	33.34±1.56	20.0±1.41	25.91±1.05	59.07±5.76	2.28±0.43
November 2023	27.99±3.86	15.91±2.64	21.02±2.87	64.81±8.97	2.15±1.04
December 2023	24.10±1.80	12.36±1.5	17.35±1.20	67.20±10.03	2.36±0.96
January 2024	21.39±2.64	8.99±1.93	14.39±1.88	58.44±12.99	2.28±0.93
February 2024	22.22±2.94	8.85±1.92	14.67±1.84	63.47±9.12	2.25±0.72
March 2024	26.93±2.98	10.89±1.34	18.04±2.08	52.85±15.81	2.16±0.94

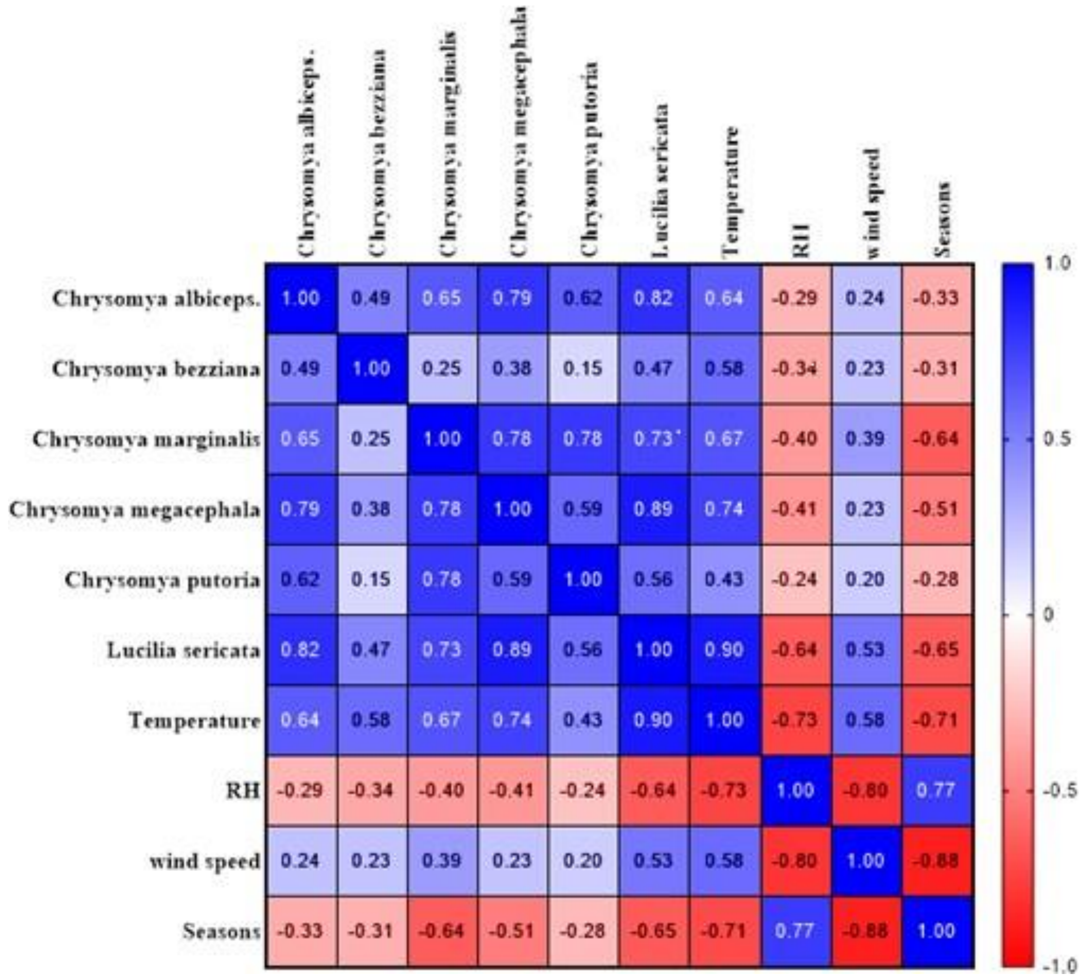

Figure 3. Correlation matrix for the different collected fly species with the different environmental conditions and seasonal variations. Correlation coefficient factor 0.5 > indicated a strong positive correlation and correlation factors < -0.5 indicated a strong negative correlation.

3.3 Relationship between bait trap type and abundance of Calliphoridae flies (host preference)

Table 4 and Figure 4 show the insect fauna collected with three types of baits during the period from April 2023 to March 2024 revealed 6 species, represented by 558 individuals. All species were attracted to fish trap and represented by 300 individuals (53.76%) of the total number of collected insects. Meanwhile, the files collected from liver traps revealed 4 species representing by 129 individuals (23.1%) of the total number of collected insects, *Ch. bezziana* and *Ch. albiceps* not attracted to liver trap. Also, all species were collected with meat trap except *Ch. putoria* with total number 129 individuals (23.1%) of the total number of collected insects. Comparing the three bait types in attracting scavenger insects, the fish traps were the most attractive to calliphoridae flies, while the meat traps are equal to liver traps attraction to different species. Regarding host preference, the data reported that, *Ch. putoria* preferred bait fish only by 100.0%, followed by *Ch. bezziana* 76.5%, *Ch. megacephala* 55.6% and *L. sericata* 48.6%. Moreover, *Ch. bezziana* prefers fish and meat baits and dose not prefer liver baits.

Table 4. Preference Calliphoridae species for three bait (host) traps in (Hihya), Al-Sharqia Governorate, during the period from April 2023 to March 2024

Bait traps	Number of Species collected (%)						Total No.	%
	Chrysomya albiceps	***Chrysomya bezziana***	***Chrysomya putoria***	***Chrysomya marginalis***	***Chrysomya megacephala***	***Lucilia sericata***		
Fish	30 (46.9)	13 (76.5)	12 (100.0)	44 (51.2)	133 (55.6)	68 (48.6)	**300**	**53.8**
Liver	13 (20.3)	00 (0.0)	00 (0.0)	23 (26.7)	57 (23.8)	36 (25.7)	**129**	**23.1**
Meat	21 (32.8)	4 (23.5)	00 (0.0)	19 (22.1)	49 (20.5)	36 (25.7)	**129**	**23.1**
Total (%)	**64 (11.5)**	**17(3.05)**	**12 (2.2)**	**86 (15.4)**	**239 (42.8)**	**140 (25.1)**	**558**	**100**

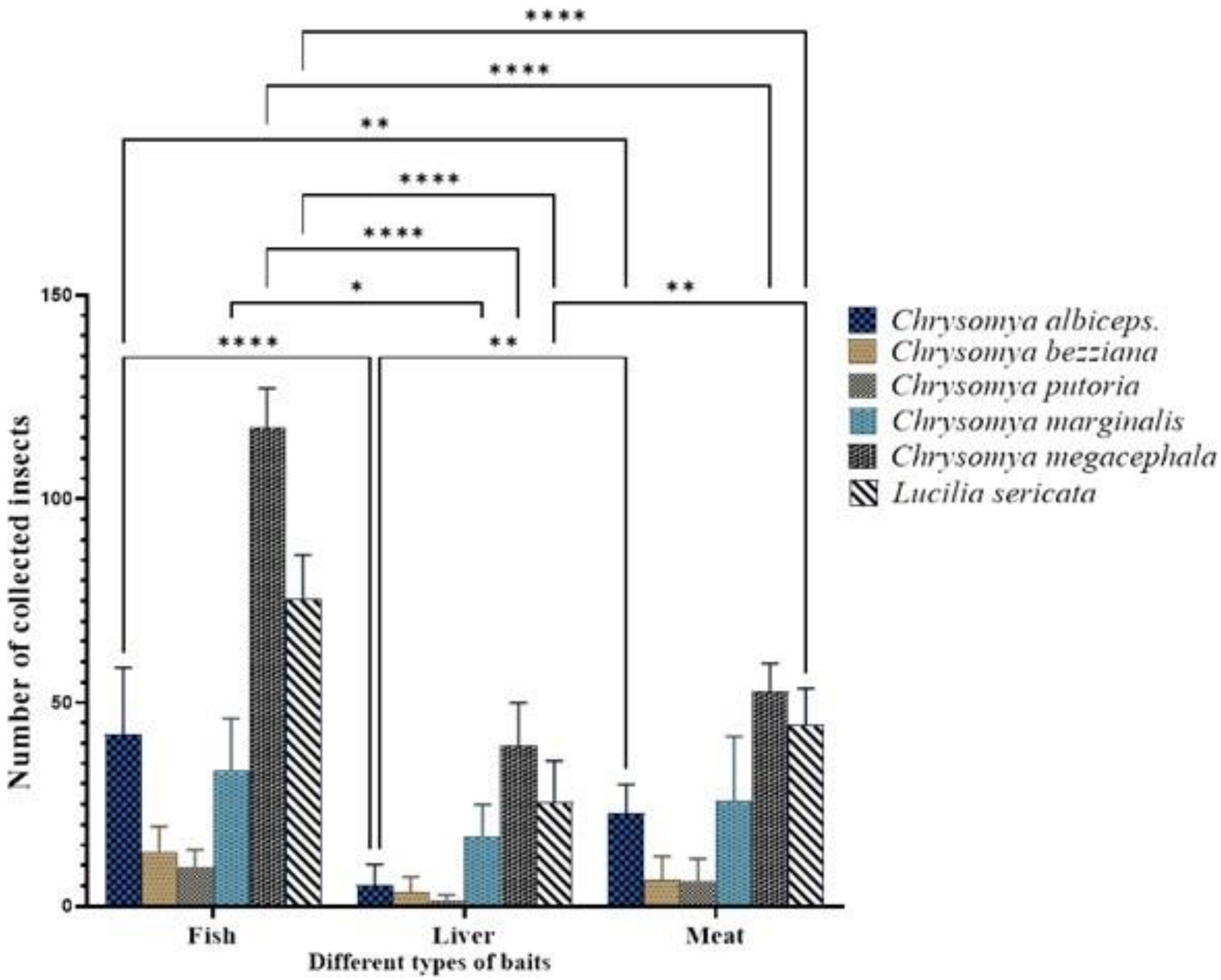

Figure 4. Effect of the different types of baits on the prevalence and species of the collected flies Values are the mean ± S.E.M. *p < 0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

3.4 Isolation and identification of bacteria from the surface of insects

In the current study 92 bacterial isolates were collected from samples and grown on different media, all isolates were grown normally on nutrient agar, while 61 able to grow on Macconkey agar medium. According to gram stain reaction 61 isolates were negatively reactive with gram stain while others 31gram positive (Figure 5).

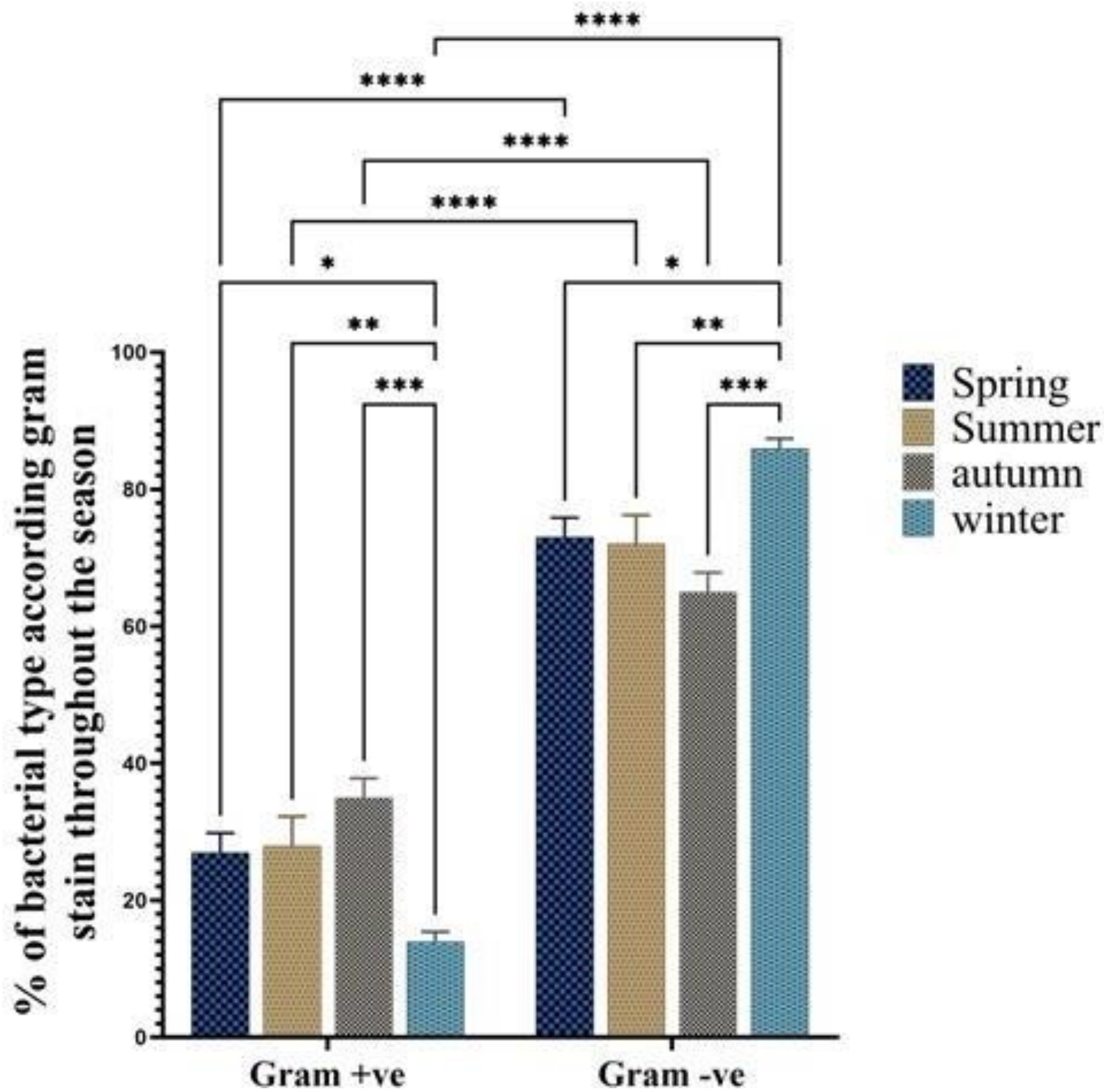

Figure 5. Classification of specimens investigated according to Gram type. Values are the mean ± S.E.M. *p < 0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

The initial identification of the bacterial isolates (92 isolates) was carried out according to the differential media assigned to each bacterial species, and some biochemical properties were studied (Table 5). The results showed five bacterial groups: *Escherichia sp.* (48 isolates), *Pseudomonas sp.* (7 isolates) *Staphylococcus sp.* (18 isolates), *Bacillus sp.* (13 isolates), and *Salmonella sp.* (6 isolates), and their percentages were 52%, 8%, 19%, 14%, and 7%, respectively.

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Table 5. Primary identification of bacterial isolates.

No.	Specimen Code	Nutrient Agar	Macconkey Agar	Mannitol Agar	EBM Agar	Cetrimide Agar	RVS Broth media	Macconkey Broth	XLD Test	Gram Stain	KOH Test	Oxidase Test	Coagulase Test
1	A1-1	+	+	-	+	-	-	+	-	-	+	-	-
2	A1-2	+	-	+	-	-	-	-	-	+	-	-	-
3	B	+	+	-	+	-	-	+	-	-	+	-	-
4	J1-1	+	-	+	-	-	-	-	-	+	-	-	+
5	J1-2	+	+	-	+	-	-	+	-	-	+	-	-
6	Q	+	+	-	+	-	-	+	-	-	+	-	-
7	Y1-1	+	+	-	+	-	-	+	-	-	+	-	-
8	Y1-2	+	-	-	-	+	-	-	-	-	+	+	-
9	Z	+	+	-	+	-	-	+	-	-	+	-	-
10	A2-1	+	-	+	-	-	-	-	-	+	-	-	-
11	A2-2	+	+	-	+	-	-	+	-	-	+	-	-
12	M2-1	+	+	-	+	-	-	+	-	-	+	-	-
13	M2-2	+	-	+	-	-	-	-	-	+	-	-	+
14	N2	+	+	-	+	-	-	+	-	-	+	-	-
15	O2-1	+	+	-	+	-	-	+	-	-	+	-	-
16	O2-2	+	-	-	-	+	-	-	-	-	+	+	-
17	B3	+	+	-	+	-	-	+	-	-	+	-	-
18	C3-1	+	-	+	-	-	-	-	-	+	-	-	+
19	C3-2	+	+	-	+	-	-	+	-	-	+	-	-
20	D3-1	+	-	+	-	-	-	-	-	+	-	-	-
21	D3-2	+	+	-	+	-	-	+	-	-	+	-	-
22	1-1	+	+	-	+	-	-	+	-	-	+	-	-
23	1-2	+	-	-	-	-	+	-	+	-	+	-	-
24	2-1	+	+	-	+	-	-	+	-	-	+	-	-
25	2-2	+	-	+	-	-	-	-	-	+	-	-	-
26	3-1	+	-	+	-	-	-	-	-	+	-	-	+
27	3-2	+	+	-	+	-	-	+	-	-	+	-	-
28	3-3	+	-	-	-	-	+	-	+	-	+	-	-
29	15	+	+	-	+	-	-	+	-	-	+	-	-
30	16-1	+	+	-	+	-	-	+	-	-	+	-	-
31	16-2	+	-	+	-	-	-	-	-	+	-	-	-
32	26-1	+	-	-	-	-	+	-	+	-	+	-	-
33	26-2	+	+	-	+	-	-	+	-	-	+	-	-
34	31-1	+	-	+	-	-	-	-	-	+	-	-	+
35	31-2	+	+	-	+	-	-	+	-	-	+	-	-

36	**32**	+	+	-	+	-	-	+	-	-	+	-	-
37	**33-1**	+	-	+	-	-	-	-	-	+	-	-	-
38	**33-2**	+	-	-	-	+	-	-	-	-	+	+	-
39	**33-3**	+	+	-	+	-	-	+	-	-	+	-	-
40	**44-1**	+	+	-	+	-	-	+	-	-	+	-	-
41	**44-2**	+	-	+	-	-	-	-	-	+	-	-	-
42	**45**	+	+	-	+	-	-	+	-	-	+	-	-
43	**46-1**	+	-	+	-	-	-	-	-	+	-	-	+
44	**46-2**	+	+	-	+	-	-	+	-	-	+	-	-
45	**46-3**	+	-	-	-	+	-	-	-	-	+	+	-
46	**59-1**	+	-	+	-	-	-	-	-	+	-	-	-
47	**59-2**	+	+	-	+	-	-	+	-	-	+	-	-
48	**60-1**	+	+	-	+	-	-	+	-	-	+	-	-
49	**60-2**	+	-	+	-	-	-	-	-	+	-	-	+
50	**61**	+	+	-	+	-	-	+	-	-	+	-	-
51	**74-1**	+	+	-	+	-	-	+	-	-	+	-	-
52	**74-2**	+	-	+	-	-	-	-	-	+	-	-	-
53	**75-1**	+	-	+	-	-	-	-	-	+	-	-	-
54	**75-2**	+	+	-	+	-	-	+	-	-	+	-	-
55	**75-3**	+	-	+	-	-	-	-	-	+	-	-	+
56	**84**	+	+	-	+	-	-	+	-	-	+	-	-
57	**88**	+	+	-	+	-	-	+	-	-	+	-	-
58	**93**	+	+	-	+	-	-	+	-	-	+	-	-
59	**94-1**	+	+	-	+	-	-	+	-	-	+	-	-
60	**94-2**	+	-	+	-	-	-	-	-	+	-	-	-
61	**94-3**	+	-	-	-	-	+	-	+	-	+	-	-
62	**95-1**	+	+	-	+	-	-	+	-	-	+	-	-
63	**95-2**	+	-	+	-	-	-	-	-	+	-	-	+
64	**105-1**	+	+	-	+	-	-	+	-	-	+	-	-
65	**105-2**	+	-	-	-	-	+	-	+	-	+	-	-
66	**106-1**	+	-	-	-	-	+	-	+	-	+	-	-
67	**106-2**	+	-	+	-	-	-	-	-	+	-	-	-
68	**106-3**	+	+	-	+	-	-	+	-	-	+	-	-
69	**107-1**	+	-	+	-	-	-	-	-	+	-	-	+
70	**107-2**	+	+	-	+	-	-	+	-	-	+	-	-
71	**120-1**	+	+	-	+	-	-	+	-	-	+	-	-
72	**120-2**	+	-	+	-	-	-	-	-	+	-	-	+
73	**121-1**	+	-	+	-	-	-	-	-	+	-	-	-
74	**121-2**	+	-	+	-	-	-	-	-	+	-	-	+
75	**121-3**	+	+	-	+	-	-	+	-	-	+	-	-
76	**122-1**	+	-	-	-	+	-	-	-	-	+	+	-
77	**122-2**	+	+	-	+	-	-	+	-	-	+	-	-
78	**135-1**	+	+	-	+	-	-	+	-	-	+	-	-

79	**135-2**	+	-	+	-	-	-	-	-	+	-	-	+
80	**139-1**	+	-	+	-	-	-	-	-	+	-	-	+
81	**139-2**	+	+	-	+	-	-	+	-	-	+	-	-
82	**139-3**	+	-	-	-	+	-	-	-	-	+	+	-
83	**145**	+	+	-	+	-	-	+	-	-	+	-	-
84	**146-1**	+	-	+	-	-	-	-	-	+	-	-	+
85	**146-2**	+	+	-	+	-	-	+	-	-	+	-	-
86	**147-1**	+	-	-	-	+	-	-	-	-	+	+	-
87	**147-2**	+	+	-	+	-	-	+	-	-	+	-	-
88	**147-3**	+	-	+	-	-	-	-	-	+	-	-	+
89	**159-1**	+	-	+	-	-	-	-	-	+	-	-	+
90	**159-2**	+	+	-	+	-	-	+	-	-	+	-	-
91	**160-1**	+	+	-	+	-	-	+	-	-	+	-	-
92	**160-2**	+	-	+	-	-	-	-	-	+	-	-	+

(+) Positive results (-) Negative results

In this study, the first identification was confirmed using the VITEK2 system for five bacterial groups, as it uses a number of biochemical tests and comparing them to a standard library. The results showed that these groups correspond to five bacterial organisms and they were defined as follows: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella typhimurium.* At the end of this study, a correlation was made between the types of insects collected from the different baits and the types of bacteria isolated from the surface of these insects. The results showed that the *Escherichia coli* strain represented the highest percentage of strains isolated from the surface of insects collected from fish, liver, and meat baits, respectively (Figure 6). The results also showed that the most common insect species carrying microbes were *Chrysomya megacephala*, *Lucilia sericata*, *Chrysomya marginalis,* and *Chrysomya albiceps*, respectively.

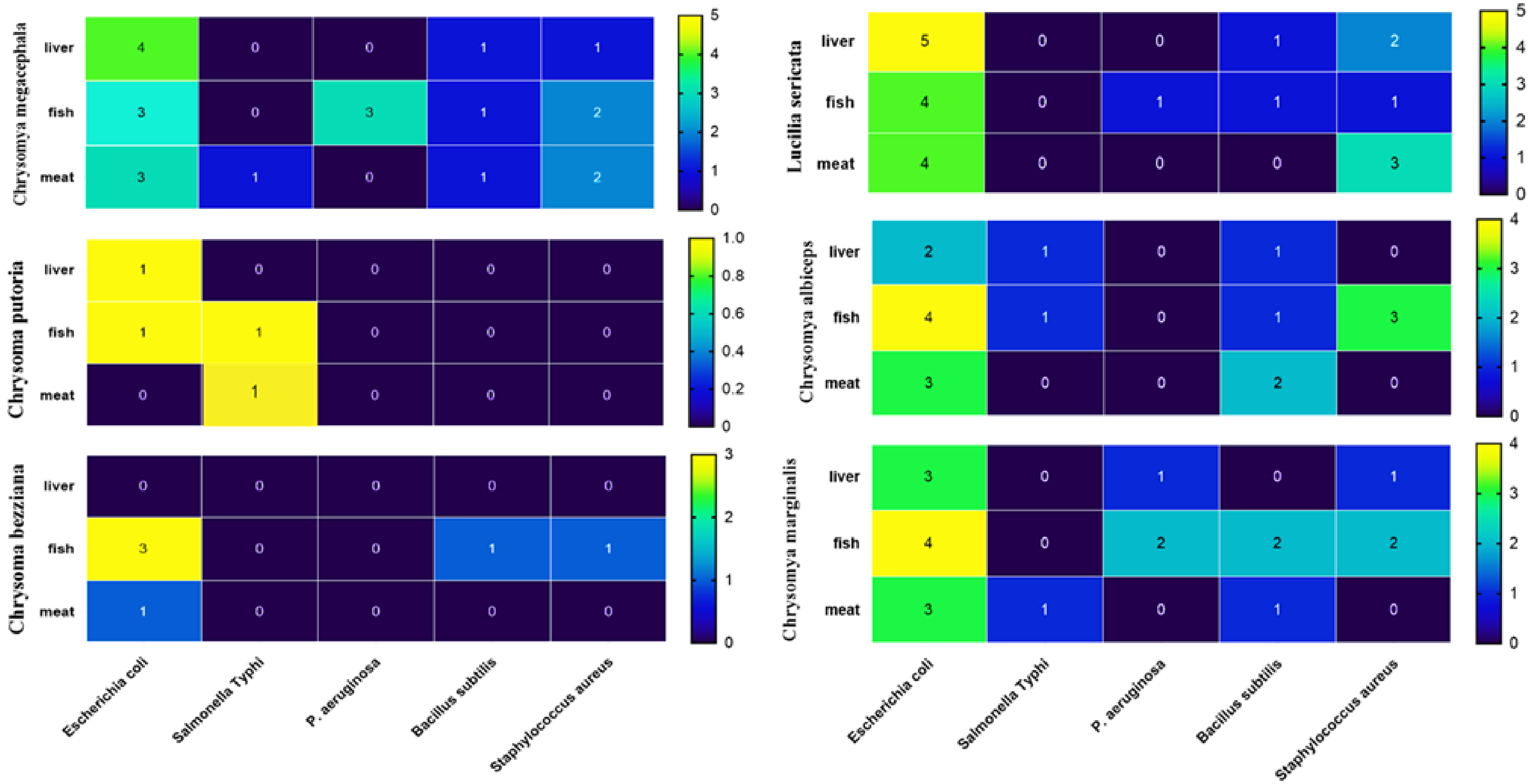

Figure 6. A heat map illustrated the correlation between different types of bait with the type of the bacterial isolates.

4. Discussion

This is the first study of its kind to examine the seasonal abundance and diversity of Calliphoridae flies using three types of baits (fish, meat and liver) in Egypt, in addition to isolating and identifying the pathogenic bacteria carried by these insects. Calliphoridae (blow flies) comprise a dangerous group of non-biting flies that transmit pathogens via mechanical transmission (Kraszewska *et al*. 2021, Dar and Mir 2024).

In the current study, during the period from April 2023 to March 2024, six species belong to Calliphoridae family and two subfamilies Chrysomyinae and Luciliinae were identified within two genera *Chrysomya* and *Lucilia*. The subfamily Chrysomyinae was more diverse with five species (*Ch. albiceps*, *Ch. marginalis*, *Ch. putoria*, *Ch. megacephala*, *Ch. bezziana*) than Luciliinae with one species (*L. sericata*). Furthermore, *Ch. megacephala* was abundant followed by *L. sericata*, *Ch. marginalis* and *Ch. albiceps*, while *Ch. putoria* and *Ch. bezziana* were less abundant. Temperature and humidity played an important role in the abundance and diversity of Calliphoridae flies during the current study. The relative abundance, diversity and seasonality of Chrysomyinae and Luciliinae were greater in summer season (July, August, September) compared to the other seasons and the same subfamilies recorded lower relative abundance and diversity in winter (Jan., Feb., Mar.).

Our results agree to some extent with previous results obtained in Egypt, with the difference in methods and baits used in collecting Calliphirodae flies, as follow; Tantawi *et al.* (2018) reported that (*Calliphora vicina*, *Ch. albiceps*, *Ch. megacephala*, and *L. sericata*) were the most common and abundant insects collected from 86.6% of the human corpses and infested corpses in all seasons and habitats in Kom El-Deka, Alexandria, Egypt. *Ch. megacephala* and *L. sericata*, were able to invade each 20% of the corpses where they acted as primary flies, and these species were abundant in summer season. Hammad *et al.* (2019) after using baits trap of yeast, sugar, salted fish, shrimp paste and fresh liver to study abundance and diversity of medical flies along Wadi El-Rayan Protected Area Egypt, found that Calliphoridae was the most abundant and diversify family during the study period, the most dominant fly's species were *Musca domestica* and *Ch. megacephala*. Seasonally, flies thrive in summer, while winter is the least abundant season.

Farag *et al.* (2021) studied the decomposition process of rat carrions and the diversity and succession pattern of the forensically important arthropods fauna that colonize these carrions in Al-Sharqia Governorate, Egypt, with Latitude: 30° 35′ 15″ N and Longitude: 31° 30′ 07″ E. Although these research scientists designed their experiments in the same governorate and very close to the place where we conducted our experiments, they only obtained two species of the Calliforidae flies (*C. bezziana* and *C. albiceps*). This difference may be due to the method used to collect insects or the different nature of the place from which the collection was done. AbdAllah *et al*. (2023) identified various species of synanthropic filth flies in the selected animal rearing stations in Assiut Governorate, Upper Egypt from July 2020 to June 2021. They recorded that, family Muscidae was the most prevalent family in all the studied stations, while the least abundant was family Calliphoridae (*Ca. vicina*, *Ch. megacephala*, and *Lu. sericata*). Regarding seasonal changes, they found that summer and spring seasons witnessed the highest incidence rates of flies followed by autumn, meanwhile the winter showed the lowest flies' abundance. The type of bait used in the current study had a significant impact on the abundance and diversity of Calliphoridae flies. All species collected in this study were attracted to the fish trap, and the *Ch. megacephala* fly was the most attracted to the fish bait, followed by *Lu. sericata*, while the *Ch. bezziana* and *Ch. putoria* flies were not attracted to the liver bait, also, *Ch. putoria* not attracted to meat traps. Worldwide, several studies have surveyed calliphorid flies using baited traps or animal carcasses (Brundage *et al*. 2011, Farinha *et al*. 2014, Weidner *et al*. 2015, 2017, LeBlanc *et al*. 2021).

These studies have contributed significantly to our knowledge of the distribution of blowfly species in different ecological regions. Jeong *et al*. (2022) used pork kidney bait traps to study the biodiversity and seasonal distribution of blowflies at the University of Tennessee, USA. After a 14-month-long blowfly survey they collected 13 species from 7 genera; *Phormia regina* (Meigen), *Lu.coeruleiviridis* and *Ch. megacephala* were the predominant species, representing of collections, respectively. *P. regina* being the most common species in spring and summer, respectively and *L. coeruleiviridis* being the most common species in fall. Barkah *et al*. (2017) used Sardine fish bait traps to determine the diversity and monthly abundance of important blowflies at the University of Tripoli, Libya during the period from Jan. 2016 to Feb. 2017.

They recorded six calliphorid species: *Ch. megacephala*, *Ch. albiceps*, *Lu. sericata*, *Lu. cuprina*, *Ca. vicina* and *Pollenia* sp, but the most abundant species was *Ch. megacephala*. They also found that all calliphoridae species show a similar seasonal trend with high abundances in the temperate and cold months while no individuals were captured during July and August. The results obtained by Al-Qurashi (2016) are consistent with our results when it was used Sheep liver bait traps for investigated the abundance of two blowflies' species from Feb. 2013 to Jan. 2014 in Jeddah City, Saudi Arabia. The results showed that the blow fly *Ch. megacephala* was the most abundant in all the sites compared to *Ch. marginalis*. The seasonal activity peak of the *C. megacephala* fly was in the winter, where the average temperature reaches a maximum of 30.8 and a minimum of 20.9, the activity of these insects was less in the summer, as the maximum temperature reached 37.2 °C and the minimum was 28.4 °C. The seasonal activity of insects in Jeddah city differs with our results obtained in the current study because of the difference in climatic factors in the four seasons between Egypt and Saudi Arabia. Phasuk *et al*. (2013) used four bait traps (raw chicken meat mixed with cooked rice, raw pork small intestine, raw shishamo fish and fresh squid) to assess the seasonality of blow flies in three tropical, urban parks in Bangkok, Thailand. They identified five species from three genera, *Ch. megacephala* was more abundant followed by *Ch. rufifacies*.

The highest numbers of *Ch. megacephala* were captured in January while *Ch. rufifacies* reached their peak in September. Anderson and Galloway, (2023) using beef liver and overly ripe bananas bait traps to study Species composition and seasonal abundance of synanthropic flies (Diptera: Calliphoridae) in the Winnipeg area, Manitoba, Canada and they collected five species, *Cynomya cadaverina*, *Protophormia terraenovae*, *Phormia regina*, *Lu. illustris*, and *Lu. sericata.* Augul *et al*. (2013) studied the prevalence of the adults of blow flies that attracted to the yellow sticky traps and the prevalence of these species in some Iraq governorates, they recorded that *Ch. albiceps* was the most abundant species caught in all studying governorates followed by *Lu. sericata*. Differences in the species composition and seasonality abundance between the current study and previously published works may have resulted from differences in the methods used, such as baited traps used to attract insects, nature of the geographical region including the habitats, type of soil, and vegetation and the climatic conditions of each region.

The seasonal prevalence of Calliphoridae flies in Sharkia Governorate has significant implications for public health and veterinary concerns. These flies are known to transmit various bacterial pathogens, including *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*, among others (Khamesipour *et al*. 2018). The result of the current investigation revealed that the different types of the isolated bacteria could be categorized in 66 % was gram –ve and 34% was gram +ve, moreover, the abundance of the bacterial occurrence was significantly differed from season to another season which was in accordance to **(**Khamesipour *et al*., 2018**)**. The results showed that the *Escherichia coli* strain represented the highest percentage of strains isolated from the surface of insects. Interestingly the high abundance of these flies during the summer months increases the risk of disease transmission to humans and animals (Amin *et al*. 2019). Moreover, the occurrence of myiasis cases in the region is closely linked to the seasonal prevalence of Calliphoridae flies (El-Tantawy 2015).

5. Conclusion

Based on the previous findings it could be concluded that the most prevalent type of flies collected from Hihya was *Chrysomya megacephala.* Also, the most suitable bait for collecting this fly was fish bait and the best season for its collection was summer in August. Furthermore, the higher abundance of the bacteria was gram –ve and positively correlated with the temperature. Further future investigations are needed to endorse the clinical importance of each fly regarding human and livestock health problem.

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