The Comparative Effectiveness of Adult Mosquito Sampling Methods Dealing With Odor-baited Resting Box Traps in a Malaria-endemic Area in Southern Iran

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Abstract

Background

Iran is under threat of a potential outbreak of mosquito-borne diseases, such as malaria, Dengue fever, Chikungunya, and Zika. The study aimed to determine the efficiency and sustainability of some adult mosquito sampling methods for designing effective entomological surveillance systems in a malaria endemic area.

Methods

Different rates of tap water, sugar, and yeast (*Saccharomyces cerevisiae*) were mixed to provide CO₂. *Anopheles stephensi* and *Culex pipiens* Bandar Abbas strains were reared in the insectary and used for tests. On a lab scale, CO₂ orientation experiments were performed using a Y-tube olfactometer on the insectary mosquito strains. In the field trial, human landing catches (HLC), artificial pit shelter (APS), CO₂-baited trap (CO₂-BT), human and cow odor baited resting boxes (HOBT, COBT), cow urine baited trap (CUBT), and colored un-baited box (UB) were studied in Bandar Abbas, Hormozgan Province, south of Iran. Mean densities of *An. stephensi* and *Cx. pipiens* insectary strains, which oriented to CO₂ as flowrate of 170, and 300 mL/minute was significantly higher compared to the control group ($P < 0.05$). The black un-baited inbox resting traps significantly more collected wild mosquito species compared to others colored UB ($P \leq 0.05$).

Results

A total of 2722 collected mosquitoes comprises *Culex pipiens* (48.56%), *Anopheles fluviatilis s.l.* (14.21%), *An. stephensi* (11.68%), *Cx. theileri* (9.95%), *Aedes caspius* (7.01%), *An. dthali* (6.79%), *An. culicifacies s.l.*, *An. pulcherrimus*, *An. sergentii*, *An. superpictus s.l.*, *Cx. sitiens* and, *Ae. caspius* less than 1%. *Anopheles stephensi* and, *An. sergentii* were most collected in CUBT. *Anopheles fluviatilis s.l.* and *Ae. caspius* were most found in HLC. *Anopheles dthali*, *Cx. pipiens* and, *Cx. theileri* were most abundant in APS.

Conclusions

Black CUBT and APS methods can be suggested as a perfect sampling strategy for malaria vectors surveillance. APS, and HLC methods were found useful to entomological surveillance systems for arboviral and filarial vector-borne diseases. Further modified sampling methods should be devoted to identify more effective sampling methods.

Background

Blood-sucking mosquitoes have been responsible for a series of diseases to humans, such as Malaria, Dengue fever, West Nile, Japanese encephalitis (JE), Zika and Yellow fever and, Chikungunya [1]. These pathogens have been spread rapidly worldwide over the last 50 years [2]. Climate change has had a huge impact on the distribution and incidence rates of arthropod-borne diseases [1]. In 2019, in 87 malaria-endemic countries, 229 million malaria cases have been estimated [3].

There are 70 species and 8 (or 12) genera of Iranian mosquitoes depending on the classification of the tribe Aedini [4]. Malaria is a major endemic infectious disease in Iran, especially in the south and southeastern provinces including the Sistan & Baluchestan, Hormozgan, and Kerman Provinces [5-10]. *Plasmodium vivax* was the causative agent of 93.75% of cases, followed by *P. falciparum* (6.25%). The 15-year-old group and elderly contained the most malaria-reported cases (66.7%) [11]. Although malaria cases have declined in Iran, imported cases from neighboring countries are still the main challenge to the national health policy [12].

Anopheles stephensi, *An. fluviatilis s.l.*, *An. culicifacies s.l.*, *An. maculipennis*, *An. superpictus s.l.*, *An. sacharovi*, and *An. dthali* are the main and *An. pulcherrimus* is considered as the probable malaria vector in Iran [13, 14]. Also, the infection of *Culex pipiens*, *Aedes caspius* and *Cx. theileri* to West Nile virus was reported from the north, northwest and, south of Iran respectively [15-17].

Many strategies have been conducted to control infectious diseases through the usage of insecticides against mosquitoes, however, the use of insecticides may result in increased levels of tolerance or resistance to pesticides [18]. Understanding the malaria transmission dynamic depends on vector behavior. The mosquitoes-borne diseases have different host feeding patterns and resting behavior (outdoor or indoor) after taking blood meals. Moreover, the microclimate can affect this habit [19]. Mosquitoes are attracted to their proper host through olfactory and visual cues. For instance, *An. gambiae s.l.* is attracted by chemicals such as ammonia, lactic acid, and carboxylic acids emanating from the human body [20]. The orientation of mosquitoes to CO₂ and heat enhances the expertise of traps for the collection of mosquitoes [21]. The mosquitoes' maxillary palp capitata peg neurons, which include Gustatory receptors (Gr1, Gr2, and Gr3) have the centric function to detect carbon dioxide [22].

Baited resting boxes have been introduced as an efficient technique for the sampling of *Anopheles* mosquitoes [23]. In a previous survey carried out by Zaim et al. (1986), the CDC light traps were more effective to collect important malaria vectors than pyrethrum space-spray catches, human and animal landing catches and, pit shelter collections. So far, odor-baited resting inbox traps have not been evaluated during malaria vector control operation in Iran [24].

Hormozgan Province is one of the most important malarious areas and malaria cases has been determined based on the national malaria surveillance system. A malaria elimination program began intending to achieve this target in Iran by 2025 [11]. There has been an excellent program since, but the continued risk of importation of malaria, Dengue, and Chikungunya cases from Pakistan and Afghanistan have a huge challenge to vector-borne diseases control.

The determination of host preference is a critical issue in the control and surveillance of blood-sucking mosquitoes; hence, we researched to extend our knowledge about the collection of mosquitoes and their response to attractants. Present research aimed at the evaluation of odor-baited resting inbox traps by testing their influence on host-seeking behaviors of mosquitoes under laboratory, and field conditions.

Methods

Study location information

This project was performed between September 2015 and September 2016 in three localities, Hormoodar (27°31'70"N; 56°31'40"E), Nian (27°50'15"N; 56°86'30"E), and Kovveh villages (27°72'81"N; 57°33'25"E), Bandar Abbas County, the capital town of Hormozgan Province, southern Iran. The villages are hillsides with a dry-warm climate and excessive humidity from June to October and the common precipitation is about seventy-five mm annually. Farmers occasionally use running water for irrigation. Many ditches, creek, waterlogged, and hoof imprints make appropriate breeding places for mosquitoes.

Rearing the species

Anopheles stephensi and *Cx. pipiens* Bandar Abbas strains have been reared in the insectary of Bandar-Abbas Health Training and at average temperature ($25 \pm 2^{\circ}\text{C}$), relative humidity ($70 \pm 10\%$), and photoperiod (10L:14D).

CO₂ production and following lab scale trial in the insectarium

We combined different rates of tap water, sugar, and yeast (*Saccharomyces cerevisiae*) to supply CO₂ in a plastic bottle (4 L) containing 2.5 L of water, based on literature [25]. In this approach, 6 concentrations of carbon dioxide had been provided fresh every day. A standard manual bubble flowmeter, Sigma-Aldrich model is used to measure the amount of CO₂. (i.e. 170, 300, 190, 250, 340, and 360 mL/min) (Table 1).

The CO₂ orientation experiments were performed using a Y-tube olfactometer, as described by Guha et al. (2014) [26] with few modifications. CO₂ was released inside the treatment arm of the Y-tube olfactometer. At the same time, nylon perforated mesh was placed in the control arm of the Y-tube olfactometer to provide the mosquitoes with free access to the external environment. The temperature and relative humidity of the experiments was maintained at $25 \pm 2^{\circ}\text{C}$ and $70 \pm 10\%$, respectively. Five replicates of each 5–7-day old adult non-blood fed female, *An. stephensi* and *Cx. pipiens* mosquitoes were exposed for each concentration and the run time for each experiment was 5 min. For each replicate, 20-25 female mosquitoes were released into mosquito introduction ports at the stem of the Y-tube olfactometer and the acclimatization time was 5 min. The airflow was maintained at 1 L/min during the experiment by a pressure regulator, which was described by Seenivasagan et al., (2012) [27]. The number of mosquitoes was counted and then recorded in each Y-tube olfactometer arm.

Table 1 CO ₂ generated from the mixture of yeast, sugar, and water in different doses				
Test No.	Yeast (g)	Sugar (g)	Water (L)	Dose of produced CO ₂ (mL/minute)
1	17.5	250	2.5	170
2	17.5	500	2.5	300
3	17.5	750	2.5	190
4	35	250	2.5	250
5	35	500	2.5	340
6	35	750	2.5	360

Sampling methods of adult mosquitoes in field trial

Artificial pit shelter

One pit shelter (120 lengths × 90 widths × 150 cm depth) has been constructed in each village [28] (Fig. 1). Mosquitoes were collected once a month using this method from each locality with a suction tube in the morning from 5:30 am to 7:30 am, released to well-labeled paper cups. The captured specimens were transported to the laboratory and identified to species based on a valid diagnostic key of Iran at the species level [29].

Human landing catches (HLC)

This process was carried out on three young volunteers at each collection site once a month, from 5 p.m. to 6 a.m. [28]. Mosquitoes seeking for hosts were collected by the aspirator and sorted by one hour intervals, well released into paper cups, labeled, transported to the laboratory, then identified based on a valid diagnostic key of Iran at the species level [29].

Odor-baited resting boxes Trap (OBT)

Mosquitoes attracted to odor hosts were collected at each locality site, once a month using black resting boxes (30 × 30 × 30 cm). Black cotton cloth material (30 × 30 cm) impregnated by sweat of the cow body (head, feet, and back) was used inside the resting boxes (RBs) as the mosquitos' attractant (Fig. 2). The

RBs had been set in the field at dusk and collected in the early morning. Mosquitoes were collected by the aspirator, transported to the laboratory, counted, and identified [29]. The same method was performed for boxes baited with human body odor (HOBT), cow odor baited resting boxes (COBT), and cow urine baited trap (CUBT). Sweat impregnated cloth has been discarded after each field trial, and the new odor-baited fabric has been used for similar experiments. Similar inbox size was used as treated with carbon dioxide generated flow rates as 170 mL/min (Table1).

Un-baited box (UB)

The similar black, blue, green, red and white cotton cloth materials used in RBs (30 × 30 ×30 cm) without any odor as the un-baited box.

Data analysis

The obtained data were entered into SPSS 19.0 (SPSS, Inc., Chicago, IL, USA) and were analyzed by One-Way ANOVA following the Tukey test (post-hoc analysis) to determine the mean differences. P-values of ≤ 0.05 were selected as a point of significant difference.

Results

Attraction of mosquitoes to different CO₂ concentrations

Mean densities of mosquito oriented to CO₂ treatment under the laboratory condition was significantly higher compared with the control and this was observed among the two combinations of sugar and yeast cultures (that mentioned in Table 1) in test numbers 1 and 2 (P < 0.05) (Table 2). However, when the efficacy of the different mixtures of sugar and yeast in attracting mosquitoes was compared, more orientation was found in the control group than treatment in tests No. 3 to 6.

Table 2
Comparison of CO₂ orientation experiments of mosquitoes under the insectary conditions.

Test No	Mean density± SE		P value	Mean density± SE <i>Cx.pipiens</i>		P value
	<i>An. stephensi</i>					
	Treatment	Control	≤ 0.05	Treatment	Control	≤ 0.05
1	14.75±0.11	10±0.11	≤ 0.05	15.25±0.12	6.25±0.16	≤ 0.05
2	11.5±0.16	10±0.14	≤ 0.05	11±0.14	7.75±0.11	≤ 0.05
3	9±0.14	11.25±0.13	≤ 0.05	8.5±0.17	11±0.19	≤ 0.05
4	7.75±0.14	13±0.17	≤ 0.05	8±0.12	11.5±0.18	≤ 0.05
5	7.75±0.13	13.25±0.19	≤ 0.05	8.25±0.13	10.5±0.14	≤ 0.05
6	7.75±0.16	13.25±0.15	≤ 0.05	7.75±0.11	9.75±0.13	≤ 0.05

Therefore, the most effective mixture should be of the lower concentration of 17.5 g of yeast plus 250 g of sugar plus 2.5-liter water, to release of CO₂ at a flow rate of 170 mL/minute (Table1).

Un-baited box traps

Table 3 shows the number and densities of mosquitoes collected in un-baited inbox traps with different colors in the field. A total of 217 mosquitoes, belonging to 3 genera and 7 species, were collected during 6 months. *Anopheles stephensi* was the most frequent species (36.8%) followed by *Cx. pipiens* (16.1), *An. dthali* (13.4), *An. fluviatilis s.l.* (13.4%), *Cx. theileri* (9.2%), *An. sergentii* (4.6%), and *Ae. caspius* (6.5%). Mean densities of female mosquitoes collected were significantly higher compared to males except for *An. sergentii* (P < 0.05) (Table 3).

Table 3

Mosquito number and densities within Un-baited traps.

Un-baited trap	<i>Ae. caspius</i>		<i>Cx. theileri</i>		<i>Cx. pipiens</i>		<i>An. sergentii</i>		<i>An. fluviatilis s.l.</i>		<i>An. dthali</i>		<i>An. stephensi</i>	
	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅
	No(D)	No(D)	No(D)	No(D)	No(D)	No(D)	No(D)	No(D)	No(D)	No(D)	No(D)	No(D)	No(D)	No(D)
Black	1(0.17)	4(0.67)	5(0.84)	3(0.5)	6(1)	9(1.5)	2(0.34)	1(0.17)	12(2)	3(0.5)	6(1)	4(0.67)	28(4.67)	8(1.34)
Green	4(0.67)	0(0)	3(0.5)	1(0.17)	7(1.17)	2(0.34)	0(0)	2(0.34)	5(0.84)	0(0)	8(1.34)	3(0.5)	19(3.17)	2(0.34)
Red	0(0)	2(0.34)	5(0.84)	0(0)	4(0.67)	1(0.17)	1(0.17)	2(0.34)	2(0.34)	1(0.17)	6(1)	1(0.17)	15(2.5)	2(0.34)
Blue	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Yellow	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(0.17)	0(0)
White	3(0.5)	0(0)	2(0.34)	1(0.17)	5(0.84)	1(0.17)	1(0.17)	1(0.17)	5	1(0.17)	1(0.17)	0	4	1(0.17)
Total	8	6	15	5	22	13	4	6	24	5	21	8	67	13
%	6.5		9.2		16.1		4.6		13.4		13.4		36.8	

In the field, 217 male and female's mosquitoes have been attracted to RBs, which, 92 (42.4%), 56 (25.8%), 42 (19.35%), 1(0.46%) and 26 (11.98%) have been collected to black, green, red, yellow and white RBs, respectively. In general, the attraction of mosquitoes was found significantly different from black and white RBs. Densities of *Anopheles* species attracted to Black colored RBs mosquito was significantly higher compared with other colored RBs ($P \leq 0.05$) (Table 3). The black un baited RBs were collected both sexes, and the most physiological stages of the females were blood-fed females followed by unfed, semi gravid plus gravid respectively.

Artificial pit shelter

A total of 920 mosquitoes were sampled using this sampling method. Among these, 332 (36.8%) were females while 588 (63.2%) were males. Out of the female samples, *Cx. pipiens* 197 (59.4%) were predominant species followed by *Cx. theileri* 59 (17.8%), *An. fluviatilis s.l.* 36(10.8%), *An. dthali* 22 (6.6%), *An. stephensi* 15 (4.5%), *An. superpictus s.l.* 2 (0.60%) and *An. sergentii* 1 (0.30%).

The number of gravid and semi gravid specimens of *An. fluviatilis s.l.*, *An. superpictus s.l.* and *An. dthali* was higher than the number of unfed and blood-fed, which proportion of gravid plus semi gravid to unfed plus fed was more than 1. In contrast, the number of gravid and semi gravid of *An. stephensi*, and *An. sergentii*, was lower than that of unfed and blood-fed ones, which that proportion was less than 1.

Human landing catches

A total of 541 adult mosquitoes were collected comprising *An. stephensi*, *An. dthali*, *An. fluviatilis s.l.*, *An. pulcherrimus*, *An. sergentii*, *An. culicifacies s.l.*, *Cx. pipiens*, *Cx. sitiens*, *Cx. theileri* and *Ae. caspius*. *Culex pipiens* (29.94%) and *Aedes caspius* (27.35%) were the most prevalent species in this method. *Anopheles species* were more collected during the second part of the night (9 pm -01 am) except *An. sergentii*, while the peak-biting activity of culicinae species, and *Ae. caspius* was in the first third of the night from 1700 to 2100 hours ($P=0.0001$) (Table 4).

Table 4

Mosquitoes collected by human landing catches based on time of sampling, Bandar Abbas, 2015-2016.

Time of sampling	Mean of temperature (°C)	Mean of humidity (%)	<i>Cx. pipiens</i>	<i>Cx. theileri</i>	<i>Cx. sitiens</i>	<i>Ae. caspius</i>	<i>An. dthali</i>	<i>An. stephensi</i>	<i>An. fluviatilis s.l.</i>	<i>An. pulcherimus</i>	<i>An. sergentii</i>	<i>An. culicifacies s.l.</i>
17-21 p.m.	26.5	59	88	13	2	78	2	14	55	0	2	0
21-01 a.m.	23.5	53	55	11	0	63	18	27	68	6	0	3
01-05 a.m.	22.5	59	59	19	6	0	7	0	0	1	1	0
Total			162 (29.94)	30 (5.54)	2 (0.36)	148 (27.35)	20 (3.69)	41 (7.57)	126 (23.29)	7 (1.29)	2 (0.36)	3 (0.55)

Cow urine baited resting trap (CUBT)

A total of 4 513 adult mosquitoes were identified comprising *Cx. pipiens* (37.03%), *An. stephensi* (19.88%), *An. fluviatilis s.l.* (14.03%), *Cx. theileri* 11.11%), *An. dthali* (9.74%), *Ae. caspius* (6.04%) and *An. sergentii* (2.14%) ($P=0.043$) (Table 5). The observation of the abdominal condition of the females is presented in

Table 6. Overall, the most of mosquitoes collected in the CUBT were un fed, followed by blood-fed, semi gravid plus gravid respectively. *Anopheles stephensi* was most collected in October, December, and April respectively while the other species have been abundant in autumn.

Sampling data of control baited trap

The control traps have been concurrently set up in the field for each test. The most collected species were *Cx. pipiens* (53.53%) followed by *An. stephensi* (21.21%) (Table 5). The abdominal condition demonstrated that more fed females of *An. stephensi* and *Cx. pipiens* collected than gravid and semi gravid (P=0.0001).

Cow odor-baited box trap (CBBT)

Seven species had been collected by cow odor-bated box traps (Table 5). Out of 391 individual mosquitoes, 36.82% had been identified as unfed females. *Culex pipiens* were most collected in April-March and December.

Table 5
The number of the attractiveness of sampled mosquitoes to cow urine odor, Bandar Abbas, 2015-2016.

Species	<i>An. stephensi</i>		<i>An. dthali</i>		<i>An. fluviatilis s.l.</i>		<i>An. sergentii</i>		<i>Cx. pipiens</i>		<i>Cx. theileri</i>		<i>Ae. caspius</i>		Total (%)
Month	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Jan	0	0	0	0	1	3	0	1	4	4	0	3	0	4	20 (3.89)
Feb	0	0	0	0	1	4	0	2	0	2	0	2	1	5	17 (3.31)
Mar	0	7	0	11	0	1	0	2	6	6	2	5	2	3	45 (8.77)
Apr	0	10	0	16	0	2	0	0	28	10	3	7	1	2	79 (15.39)
May	0	2	4	0	2	0	0	0	34	28	0	16	0	4	90 (17.54)
Jun	2	8	2	2	0	0	0	0	0	0	0	0	0	1	15 (2.92)
Jul	2	4	0	0	0	0	0	0	0	0	0	0	0	0	6 (1.16)
Aug	2	2	0	0	0	0	0	0	0	0	1	1	0	0	6 (1.16)
Sep	2	2	0	0	0	2	2	0	0	0	1	4	0	0	13 (.53)
Oct	10	15	1	2	5	17	0	1	19	7	3	1	1	2	84 (16.37)
Nov	14	20	2	6	8	22	0	1	24	14	2	4	0	3	120 (23.39)
Dec	0	0	2	2	2	2	0	2	4	0	1	1	1	1	18 (3.5)
Total (%)	32 (6.23)	70 (13.64)	11 (2.14)	39 (7.6)	19 (3.7)	53 (10.33)	2 (0.38)	9 (1.75)	119 (23.19)	71 (13.84)	13 (2.53)	44 (8.57)	6 (1.16)	25 (4.87)	513

Table 6
Abdominal condition of female collected by resting traps baited with cow urine, Bandar Abbas, 2015-2016.

Species	<i>An. stephensi</i>			<i>An. dthali</i>			<i>An. fluviatilis s.l.</i>			<i>An. sergentii</i>			<i>Cx. pipiens</i>		
Month	*U	F	HG+G	U	F	HG+G	U	F	HG+G	U	F	HG+G	U	F	HG+G
Jan	0	0	0	0	0	0	3	0	0	0	1	0	3	1	0
Feb	0	0	0	0	0	0	3	1	0	1	1	0	2	0	0
Mar	7	0	0	10	1	0	1	0	0	2	0	0	5	1	0
Apr	9	1	0	16	0	0	2	0	0	0	0	0	10	0	0
May	2	0	0	0	0	0	0	0	0	0	0	0	28	0	0
Jun	8	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Jul	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aug	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sep	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Oct	14	1	0	6	0	0	16	0	0	1	0	0	7	0	0
Nov	20	0	0	2	0	0	22	0	0	1	0	0	12	2	0
Dec	0	0	0	2	0	0	1	1	0	2	0	0	0	0	0
Total (%)	68 (28.09)	2 (0.82)	0	37 (15.28)	2 (0.82)	0	50 (20.66)	3 (1.23)	0	7 (2.89)	2 (0.82)	0	67 (27.68)	4 (1.65)	0
*U=unfed; F= fed; G=gravid; HG=half gravid															

Human odor-baited resting boxes

A total of 126 mosquitoes belong to 6 species had been collected by this method. *Cx. pipiens* (61.9%) was found prevalent species followed by *An. fluviatilis* s.l. (14.28%) (p=.0001). The majority of collected samples were unfed. *An. stephensi* was most collected in spring and the end of summer, while *Cx. pipiens* was found more in spring and early autumn and was a less common species during the cold season.

CO₂-baited resting traps

In this research, as mentioned before, to produce CO₂ as flowrate of 170 mL/minute, we used 17.5 g of yeast plus 250 g of sugar plus 2.5-liter water for attraction of mosquitoes to RBs (Table 1). A total of 2722 mosquitoes were collected by the seven sampling methods (Table 7). Among these 1322 (48.56%) were *Cx. pipiens*, 387 (14.21%) *An. fluviatilis* s.l., 318 (11.68%) *Anopheles stephensi*, 271 (9.95%) *Cx. theileri*, 191 (7.01%) *Ae. caspius*, 185 (6.79%) *An. dthali* and other species less than 1%. Out of 132 collected mosquitoes, *Cx. pipiens* was the most frequent species 48 (36.8%) followed by *An. stephensi* 42 (31.8), *An. dthali* 23 (17), and *An. fluviatilis* s.l. 19 (14%) (Table 7). The number and percentage of unfed females of mosquitoes collected by this sampling method were higher than that of blood-fed followed by semi gravid and gravid (P=0.024).

Table 7
Comparison of all sampling techniques based on collected methods, Bandar Abbas, 2015-2016

*Method Species	CUBT	CBBT	HBBT	Co ₂ -BT	U BT	APS	HLC	Total
<i>An. culicifacies s.l.</i>	0	0	0	0	4	0	3	7 (0.26)
<i>An. dthali</i>	50	13	11	23	5	63	20	185 (6.80)
<i>An. fluviatilis s.l.</i>	72	40	18	19	14	98	126	387(14.22)
<i>An. pulcherrimus</i>	0	0	0	0	2	0	7	9 (0.33)
<i>An. sergentii</i>	11	0	0	0	0	3	2	16 (0.59)
<i>An. stephensi</i>	102	57	12	42	21	43	41	318 (11.68)
<i>An. superpictus s.l.</i>	0	5	0	0	0	6	0	11 (0.40)
<i>Cx. pipiens</i>	190	248	78	48	53	543	162	1322 (48.57)
<i>Cx. theileri</i>	57	20	0	0	0	164	30	271 (9.95)
<i>Cx. sitiens</i>	0	0	3	0	0	0	2	5 (0.18)
<i>Aedes caspius</i>	31	8	4	0	0	0	148	191 (7.02)
Total	513	391	126	132	99	920	541	2722
Percent	18.00	14. 8436	4.62	4.84	3.63	33.79	19.87	100
*CBT= control baited tarp; CUBT= Cow urine baited trap; CBBT= Cow body baited trap; HBBT=Human body baited trap; Co ₂ -BT= Co ₂ -baited trap; APS= Artificial Pit shelter; HLC= Human landing catches; UBT=Un- baited trap								

APS collected 33.79%, HLC 19.87%, CUBT 18.84%, CBBT 14.36%, Co₂-BT 4.84%, HBBT 4.62%, and UBT 3.63%.

Anopheles stephensi was most collected in CUBT (n = 102, 32.07%), followed by CBBT (n = 57, 17.9%), APS (n = 43, 13.52%), CO₂-BT (n = 42, 13.21%), HLC (n = 41, 12.89%), UBT (n = 21, 6.60%) and HBBT (n = 12, 3.77%). There were statistically significant differences among the number of *An. stephensi* collected by CUBT, CBBT (P = 0.009), APS (0.007), HLC (0.005), CO₂-BT (P = 0.005), UBT (P = 0.003), and HBBT (P = 0.001). *An. fluviatilis s.l.* was most collected in HLC (n = 126, 32%), and least in UBT (n = 14, 3.61%). Significant differences were found among the number of this species collected by HLC, compared to other methods (P = 0.001).

Anopheles sergentii was most frequent in CUBT, whereas, *An. dthali* was more abundant in APS. There were significant differences among the number of *An. dthali* collected by APS, CUBT (P = 0.008), CO₂-BT (P=0.007), HLC (P=0.005), and CBBT (P = 0.001).

Culex pipiens was most abundant in APS (n = 543, 41.07%), followed by CUBT (n = 248, 14.37%), CBBT (n = 190, 18.76%), HLC (n = 162, 12.25%), HBBT (n = 78, 5.9%), UBT (53 = 21, 4%) and CO₂-BT (n = 48, 3.63%). Significant differences were found among the number of *Cx.pipiens* collected by APS, CUBT (P = 0.009), CBBT (P=0.007), HLC (P=0.005), HBBT (P = 0.005), UBT (P = 0.003), and CO₂-BT (P = 0.003). *Culex theileri* was most collected in APS (n = 126, 60.51%), and least in CBBT (n = 14, 3.61%). This species was not collected by HBBT, CO₂-BT and UBT. Significant differences were found among the number of this species collected by APS, compared to other methods (P = 0.001).

Aedes caspius was most frequent in HLC followed by CUBT, CBBT, and HBBT. There was no collection of this species by CO₂-BT, UBT, and APS. There were significant differences among the number of *Ae. caspius* collected by HLC, CUBT (P = 0.008), CBBT (P=0.007), and HBBT (P = 0.001).

Discussion

The useful mosquito trap was to be effective, non-toxic, and covers a wide area. The present investigation revealed that the attraction pattern of four anophelinae, two culicinae, and one aedeniae mosquitoes was varied and also highly depended on the type of the traps.

Anopheles stephensi is the main malaria vector in Iran, and three biological forms i.e. type, mysorensis, and intermediate of the species have been reported in the Hormozgan Province [30]. This study demonstrated that the cow urine-baited resting traps have been more attractive to *An. stephensi* than either odor-baited boxes or other methods. This observation was consistent with that of Kweka et al [23, 31], who reported a higher density of *An.arabiensis* in CUBT followed by CBBT, and UBT respectively. The efficiency of CUBT is dependent on the place of the trap in indoors and outdoors. In our study, CUBT has a longer desirable effect on *An. stephensi*, which tend to rest inside the houses and shelter. On the other hand, the proportion of gravid and semi gravid of *An. stephensi* species collected by CUBT was lower than that of unfed and blood-fed ones. It seems that this species blood meal on both humans and cows rests in shelters to complete their gonotrophic cycle. Despite the widespread distribution of LLINs and ITNs among residents in southern Iran, most of them do not use a mosquito net when they sleep. This finding could be highlighted for programs of vector control such as IRS (indoor residual spraying), which impact on endophilic vector species as well as health promotion through the distribution of ITNs and LLINs. This finding agrees with a study performed on *An stephensi* in the south and southeastern Iran [32] and also control of malaria program in Africa [33].

Anopheles fluviatilis s.l. is the malaria vector in Iran and only species T has been reported from Iran [34, 35]. So far, at least three sibling species S, T, and U have been reported in the world [36]. In our study, *An. fluviatilis s.l.* more collected by HLC followed by APS, CUBT, CBBT, CO2- Bt, HBBT, and CBT. The ratio of semi gravid plus gravid to unfed plus fed of female *An. fluviatilis s.l.* was more than one indicated exophilic behavior. Agree with our finding, Basseri et al (2010) was relieved exophilic behavior with a high anthropophagic index for *An. fluviatilis s.l.* species [32].

An. dthali has been reported as a secondary vector for malaria in Iran [37]. In our study, *An. dthali* was more abundant in artificial pit shelters, followed by CUBT, CO2-Bt, HLC, CBBT, HBBT, and CBT. The abdominal condition of the female *An. dthali* during our study shows exophilic behavior. Agree with our finding, Basseri et al (2010) was reported the exophilic behavior of *An. dthali* [32].

An. culicifascies s.l. is the main malaria vector in Iran [38]. In the present study, this species was most abundant in UBT (n=4, 57.2%) followed by HLC (n=3, 42.8%). This species was captured by UBT, and found unfed, freshly fed, semi gravid, and gravid. Although we collected a few *An. culicifascies s.l.* during of this study, based on the ratio of abdominal condition was observed both endophilic and exophilic behaviors. The same result has been reported in a previous study [32]. In our study, this species was collected in the second third of the night by HLC. Agree with our study, more biting activity of this species was observed before midnight [39]. In contrast, this species was reported as the zoophilic behavior [6, 38]. We collected a few *An. culicifascies s.l.* during of this study, so at the present, we were unable to decide about comparable behavior.

Anopheles superpictus s.l. is one of the seven species of malaria vectors in Iran [6]. This species were captured in CBBT (n=5, 45.4%) followed by APS (n=6, 54.6%). Exophilic behavior of this species collected by APS was found based on the ratio of abdominal condition. The same result has been reported by Nejati et al. (2013) [6]. We collected a few *An. superpictus s.l.* during of this study, so at the present, we were unable to decide about comparable behavior. *Anopheles superpictus s.l.* is the main vector in the Central Plateau of Iran [40]. This species had the highest abundance and distribution among *Anopheles* in Kashan County and the best sampling method for its adults was the BG trap [41, 42].

An. pulcherrimus is a suspected malaria vector in Iran [39]. Our founding relieved the more abundant of this species in UBT (n=2, 22.2%) followed by HLC (n=7, 77.8%). In our study, this species was collected in the second third of the night by HLC. Agree with our study, more biting activity of this species was found to occur before midnight [39]. Also, this species was captured by UBT, and found unfed, freshly fed, semi gravid, and gravid. Although we collected a few *An. pulcherrimus* during this study, based on the ratio of abdominal condition was observed exophilic behaviour. Agree with our study Zaim et al (1993) was reported the exophilic behaviour of this species [38]. We collected a few *An. pulcherrimus* during of this study, so at the present, we were unable to decide about comparable behavior.

In our research, the use of various sampling methods to collect some culicinae sub-family and the tribe Aedini species from indoor and outdoor places helped us to know more about the behavior diversity and densities of these species.

Culex pipiens complex includes several members, more than 75 binomial names due to the complexity of synonyms [43]. Among the species complex, *Culex pipiens*, *Cx. quinquefasciatus* and *Cx. pipiens* form molestus are the most common members of this complex and are widespread in the world [44]. The molecular and morphological studies using ITS2, COI, Ace2 cDNA genes and DNA barcoding indicated some variations of the *Culex pipiens* complex in Iran [45-47]. Because of having morphological variations among the *Cx. pipiens* complex, sometimes it is not easy to morphologically separate from *Cx. quinquefasciatus* and *Cx. torrentium* and, need to molecular assay [47]. Recently, was reported the occurrences of *Cx. pipiens pipiens* biotype *pipiens* and a hybrid between *Cx. pipiens pipiens* biotype *pipiens* and *Cx. pipiens pipiens* biotype *molestus* in Iran [48, 49]. *Culex pipiens pipiens* has a wide distribution in Iran and is also reported the steno-eurygamy, anautogeny, and ornithophilic behaviors [45, 49, 50]. West Nile and Sindbis viruses are predominantly transmitted between birds [51, 52]. This species was reported a potential enzootic and bridge vectors of SINV and WNV already circulating in Iran [53, 54]. Shahossaini et al. (2018) reported the occurrence of the West Nile virus and its subtype Kunjin in Iran [49].

In our study, more *Cx. pipiens* collected by CBBT followed by CUBT and, HBBT. It seems that, this species selected calf odor over human odor, despite similar weights and thus similar CO2 expiration rates. In agreement, Shahossaini et al. (2018) reported a high proportion of birds for *Cx. pipiens pipiens* f. *pipiens* of detected blood meal sources [49]. Similarly, Culicidae species are more oriented to mosquito Magnet traps treated with synthetic mixture than MM-X traps baited only with human odors in the field [55]. In contrast, more attraction of *Cx. quinquefasciatus* to human skin odors were reported [56]. This finding agrees with previous studies, which indicated the *Cx. pipiens* has a wide range of hosts, whereas *Culex quinquefasciatus* more attract to selected human odor over calf odor [57-62].

Culex theileri is widespread in Iran and, was reported naturally infected with *Dirofilaria immitis* and *Setaria labiatopapillosa* [63, 64]. In our study, *Cx. theileri* was most collected in APS followed by CUBT, HLC and, least in CBBT. This species was not collected during the present investigation utilizing HBBT, CO2-BT and UBT. This finding agrees with previous studies in Guilan Province, northern Iran, which indicated that this dominant species was more collected from cow shelter than human shelter using CDC light trap and Hand catch methods [65]. Shahossaini et al (2018) reported a relatively high proportion of non-human mammals for *Cx. theileri* of detected blood meal sources [49]. Prior studies carried out in Isfahan province, central Iran, was found that, *Cx. theileri* as the dominant species in both larval and adult collections with exophilic and exophagic behaviour [41, 42, 66, 67]. In Kashan County, *Cx. theileri* and *Cx. pipiens* were the only species collected using the animal-baited bed net trap [41]. In contrast, we found low abundance among culicinae species. Hormozgan has a warmer climate than Isfahan province, and perhaps may explain these unexpected results, moreover there are widespread rice fields in Isfahan, so that we cannot observe them in Hormozgan. On the other hand, a lesser abundance of this species in our study, is perhaps due to the sampling method and time of collection.

Among the species collected, *Ae. caspius* is widespread in Iran and, known to vector WNV in northwestern Iran [68]. In our research, *Aedes caspius* was most abundant in the human landing catch followed by CUBT, CBBT, and HBBT. Agree with our finding, a study performed by Nejati et al (2020) reported this species was most abundant in Malayse trap and CO2-baited trap than BG and RBs [62]. Also, in agreement, Brown et al (2018) relieved that *Ae. albopictus* most was

collected by CDC backpack aspiration, then Sticky resting bucket and, Resting bucket [69]. We observed no evidence of the occurrences of *Ae. aegypti* and *Ae. albopictus*, the important vectors of arboviral diseases in Hormozgan Province. *Aedes caspius* was found only Aedini species, which were collected in our study. In contrast, Hassandoost et al (2020), reported two species, *Ae. caspius* and *Ae. vexans* by larval collection in Hormozgan Province [70]. It should be considered, adult sampling methods in our study, may explain the variation in the results.

The human landing catch was introduced as the standard method for measuring human exposure to mosquito bites. However, this method potentially exposes individuals to a range of nuisance mosquitoes as well as mosquito-borne diseases and created bias through the sampling. On the other hand, baited resting boxes were not a suitable alternative method to collect of this species, which are more important in addressing entomological surveillance due to arbovirus transmission.

The artificial pit shelter was found more efficient than the baited resting boxes in the density and diversity of *An. fluviatilis s.l.*, *An. dthali*, *Cx. pipiens*, *Cx. theileri* and *Ae. caspius* species.

In agreement, Pombi et al. (2014) reported the low abundance of Afrotropical malaria vectors by Sticky resting box (SRB) than the pit-shelters [71]. Also, our finding agrees with a study performed on *An. arabiensis*, and *An. funestus s.l.* in south-eastern Tanzania [72], which the mean abundance of these species collected with SRB traps inside and outdoors was significantly lower than with Back Pack Aspiration and Resting Bucket traps. Overall, very low numbers of mosquitoes were capture from the RBs baited with human odour [73], an observation similar to what was found in the current study.

The proportion of gravid and semi gravid to unfed and blood-fed showed the exophilic behavior of these species. The higher density of semi-gravid and gravid mosquitoes using the APS method is useful in evaluating the oviposition behavior of malaria vectors and in determining the best control method in targeting ovipositing mosquitoes. This finding could be highlighted for programs of vector control such as larviciding and thermal fogging, which impact exophilic mosquito species.

Our finding indicated that, the case of black un-baited resting boxes was the priority for all species. Agree with our finding, Burkett et al. (1998) showing *Aedes aegypti* preferred black spots or traps [74]. Also, more attraction of *Cx. quinquefasciatus*, *Cx. sitiens*, and *An. epiroticus* to black trapping boxes were reported [75]. Khemrattrakool et al. (2019) demonstrated the most of the mosquitoes found in the back UBs were blood-fed females [76], evidence similar to what was observed in the current study. Our finding revealed that, baited RBs more collected unfed mosquitoes, which are important to identifying the parity rates and addressing the situation of malaria transmission., which are important to identifying of the parity rates and addressing the situation of malaria transmission. The urine-baited traps provide a useful alternative tool for sampling malaria vector mosquitoes. This method can also be useful to survey adult mosquito mites and fungus infection that merged from any breeding site and then collected by baited RBs.

In the laboratory and field experiments, CO₂ at a flow rate of 170 ml/min was the best dose and used to attracting mosquitoes than other doses. *Culex pipiens* was found the most frequent species followed by *An. stephensi*, *An. dthali*, and, *An. fluviatilis*. Current data approved that *Cx. pipiens* with non-human preference behavior are more targeted to traps than *Anopheles* species. In Kashan County, the best method for adult sampling of *Cx. pipiens* was BG-Sentinel trap with CO₂, followed by human baited bed net trap [41].

Agree with our finding, a study determined that the zoophilic species of *An. coustani* and, *An. pretoriensis* are more oriented to CO₂ rather than anthropophilic species [60] (Dekker and Takken 1998). In the laboratory experiments, we observed a variation among the different concentrations of CO₂ for the attractiveness of *An. stephensi* and, *Cx. pipiens*. Agree with our finding, a study determined in Tanzania showed that no correlation among the different concentrations of CO₂ for the attractiveness of *An. gambiae* and *Cx. quinquefasciatus* [25]. Carbon dioxide is an activator of mosquito host-seeking, more ever, Zöllner et al. (2004) reported increase in ambient CO₂ levels may reduce the effective range of attraction to their blood hosts [77]. Consequently, Grant et al. (1995) relieved that the variations in CO₂ concentration could affect the ability of the vector to host-seeking behavior [78]. Our finding showed that carbon dioxide raised from yeast supply was not attracted by some mosquitoes. This finding agrees with a study performed by Smallegange et al (2005), which suggested an alternative method using carbon dioxide in the field [20]. Agree with our finding, a study determined that, *An. gambiae* s.s. has been tended to CO₂, also dry ice-baited trap was well-operated rather than yeast provided CO₂ trap [60, 79].

In contrast, was reported the strong response to carbon dioxide raised from molasses supply in the open field [80]. It should be considered, that climatic factors, such as wind speed fluctuations, affected the amount of released carbon dioxide. It means CO₂ was not released at durable concentrations to attract species. In our field area, winds in the low levels become much more uniform at night to early morning, which perhaps may explain a lesser abundance of some species collected by this method.

Conclusion

Black CUBT and APS methods can be suggested as a perfect sampling strategy for malaria vectors surveillance. APS, and HLC methods were found useful to entomological surveillance systems for Arboviral and filarial vector-borne diseases. Further modified sampling methods should be devoted to identifying effective sampling methods. In conclusion, the cow urine-baited RBs and Artificial pit shelters can be considered as a specific-species sampling for malaria vector surveillance systems, Also, the human-baited double net trap, could be evaluated compared with human landing catches, for estimating human-biting rates of arboviral and filarial vector-borne diseases. On the other hand, to the monitoring of larval stage and container-breeding species, using ovitrap is suggested.

Declarations

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Ethics approval and consent to participate

This study was approved by the ethical committee of Tehran University of Medical Sciences also was conducted by the national norms and standards for conducting medical research in Iran (No. 5798).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SHM, and MMS developed the study concept, design, revised and edited the manuscript. YS collected the data, did the laboratory work, analyzed and wrote the manuscript. TSA, AR, RS, MK, AR, SF, and NS conceived and designed the experiments, performed the experiments, wrote the paper. All authors read and approved the final manuscript.

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Figures

Figure 1

Mosquito collection by Pit shelter method, Bandar Abbas, Hormozghan Province, 2015-2016.

Figure 2

Human odor baited resting box in the field, Bandar Abbas, Hormozgan Province, 2015-2016.

Supplementary Files

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