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Laboratory Evaluation of Some Local Components as Attractants to the Mosquito, *Culex pipiens* Females

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ABSTRACT

Culex pipiens is a cosmopolitan mosquito species and is an important vector for periodic lymphatic filariasis, West Nile virus, and Rift Valley fever. Using newer technologies like the application of semiochemical-baited traps for mass trapping or killing of adult females of mosquito under integrated pest management (IPM) programs have been encouraged instead of intensive using of chemical insecticides to avoid hazardous effects to human, animals, and livestock in addition to environmental problems. The present study focused on the evaluation of the efficacy of attractive traps in trapping the mosquito, *Culex pipiens* females under laboratory conditions. The traps were supplied with some of safe components such as (1) sucrose, molasses and CAPL* (Central Agricultural Pesticides Laboratory) alone and mixed with Baker's yeast granules that cause releasing of CO_2 as an attractive material and (2) ammonium hydroxide (33%) and lactic acid alone and mixed with molasses or sucrose. The obtained results showed that the sugary solutions (sucrose, molasses, and CAPL* solutions) alone had lower attractive effect (46.7- 72.0% attraction after two days exposure) as compared with those that were mixed with Baker's yeast granules (92-100% attraction). In addition CO_2 emanation showed attraction and killing effects on tested mosquitoes. Ammonium hydroxide (33%) exhibited high attraction effect at low dilution (1 µl/100 ml water attracted 100% of mosquitoes after two days exposure) while lactic acid was weak attractant (1 µl/100 ml water attracted only 44% of mosquitoes). So lactic acid (5 µl/100 ml water) attraction (60% after two days) was augmented by adding of 50 gm molasses and 50 gm sucrose (96% and 92% attraction, respectively at day 2 post-exposure). It can be concluded that solutions showed ≥ 90% attraction after two days exposure (sucrose + yeast, molasses + yeast, CAPL* + yeast, ammonium hydroxide (33%) at 1 µl and lactic acid at 5 µl mixed with sucrose or molasses) are considered attractive materials, but for control purposes, solutions which cause complete death for the attracted mosquitoes are preferred such as sucrose + yeast, molasses + yeast, and CAPL* + yeast. However, further intensive studies are needed to test the efficacy of such materials as control agents under field conditions.

INTRODUCTION

Vector-borne diseases affect nearly two-thirds of the human population in the world, mostly in developing countries especially the sub-Saharan region and kill millions annually Mosquitoes (Diptera: Culicidae) are probably the most medically important group of arthropods worldwide because of the ability of some species to transmit a wide diversity of ARBO-viruses (Arthropod-Borne viruses) that cause diseases in humans, animals and livestock (Gratz, 2007 and Clements, 2012), causing major health issues in some parts of the world. Among the approximately 3500 mosquito species currently recognized worldwide (Harbach and Howard, 2007 and Harbach, 2015) only a small number play a primary role in the transmission of arboviruses. The species that fulfills this role tends to have adopted a degree of anthropophilic behavior and occurs at high abundance and in close proximity to the susceptible hosts, primarily through exploitation of larval development sites created by humans.

Culex pipiens Linnaeus (Diptera: Culicidae) is a cosmopolitan mosquito species distributed worldwide, with the exception of Australia and Antarctica (Farajollahi *et al.,* 2011). *Culex pipiens* is an important vector for periodic lymphatic filariasis, West Nile virus, and Rift Valley fever (Calisher, 1994; Farid *et al.,* 2001; Kramer and Ebel, 2003; Farajollahi *et al.*, 2011; Abdel-Hamid *et al*., 2013 and Kenawy *et al*., 2018).

Vector control is a very integral part of the current global strategy for the control of mosquito-borne diseases (WHO, 2009). Although chemical control was highly efficient against the target species, it is facing numerous threats due to lack of selectivity, accumulation of residues in the environment and the development of resistance strains (Severini *et al.,* 1993). Other undesirable effects include hazardous effects against non-target animals, environmental problems and human health concerns (Liu *et al.,* 2006). The search for alternative pesticides and control measures that pose no risk or posing minimal risk to human health and the environment is of great interest from the preventive medicine point of view (Fatope *et al.,* 1993). Using newer technologies like application of semiochemical baited traps for mass trapping or killing of adult mosquitoes under integrated pest management (IPM) programs targeting mosquitoes have been encouraged (Kline, 2006 & 2007).

The host-finding by mosquitoes is a critical component of survival for most species and much researches is focused on the cues used for host location. Mosquito feeding behavior is a key determinant of mosquito-borne disease transmission (Ohm 2018). They find their human and other blood hosts primarily by identifying their characteristic odor profiles. Using their olfactory organs, the mosquitoes detect compounds present in human breath like CO_2, sweat and skins like ammonia, lactic acid and different carboxylic acids (Costantini *et al.,* 1996; Spitzen *et al.,* 2008 and Sukumaran 2016), and use these as cues to locate and obtain blood from the humans (Takken, 1991; Costantini *et al.,* 1996; Takken and Knols, 1999 and Okumu *et al.* 2010). Of the many cues involved, the host odor is generally considered the most important. Although mosquitoes feed on a broad range of hosts, humans have received the most attention (Steib *et al.,* 2001). Variations in the body odor of human individuals determine the differences in their attractiveness to mosquitoes (Sukumaran, 2016). Considerable researches have been conducted on odors produced by humans that elicit attraction in mosquitoes with emphasis on compounds from sweat and skin (Okumu *et al.* 2010 and Smallegange *et al,* 2010).

Among the attractive materials, sugars which are the smallest carbohydrates containing five to six carbon atoms, hydrogen and oxygen and yeast (*Saccharomyces cerevisiae*) which is a single-celled organism that feeds on glucose,

and through fermentation converts carbohydrates to alcohols while releasing carbon dioxide (CO_2) which is attractive to mosquitoes. Therefore, a solution containing sugar and yeast can be used as baits (Abdon-Liwanag and Tansengco, 2015).

It has been demonstrated that CO_2, ammonia and the lactic acid act as potential attractants for *Aedes* and *Anopheles* mosquitoes (Costantini *et al.*, 1996; Geier *et al.,* 1996 & 2000; Braks *et al.*, 2001 and Saitoh *et al.,* 2004). Attractant chemicals from host skin emanations appeared to provide the most immediate promise for use in traps. The present study aimed at determining the attraction efficacy of traps baited with some types of semiochemical attractants of natural and synthetic origins to trap the mosquito, *Culex pipiens* females under laboratory conditions.

MATERIALS AND METHODS

Test Mosquito:

Culex pipiens, the common and medically important species as vector of Rift Valley fever, West Nile virus and lymphatic filariasis in Egypt (Taylor *et al*., 1956; Hoogstraal *et al.,* 1979 and Abdel-Hamid et *al.,* 2011) was used in this study. Larvae were collected by dipping (using a white dipper with extendable handle) from stagnant breeding water at Al-Marg city (latitude: 030º010'296"N and longitude: 031º023' 088" E), Cairo Governorate. The collected larvae were transferred in jars to the rearing insectary of the Mosquito Research Department, Research Institute of Medical Entomology.

Rearing of *Culex pipiens*:

A colony of *Cx. pipiens* was established and maintained at 27 ± 2 °C temperature, 70 ± 10% RH and 12: 12 light and dark photoperiod in a laboratory where no exposure to any insecticides. The larvae were transferred into white enamel plates and fed on a mixture of yeast granules and grinded rusk powder in a ratio of 1: 2, respectively, sprinkled daily on the water surface until pupation. On each alternate day, the water from the culture tray was changed . The pupae were collected daily by a wide-mouth Pasteur pipette then placed in plastic bowls half-filled with water. The plastic bowls with pupae were placed in a wooden-framed cage (30*30*30 cm) for adult emergence. Male and female adults after emergence were daily fed on 10% sucrose solution soaked in cotton pads. Females were periodically offered blood of Pigeon. Suitable bowls for egg-laying were placed in the cages and egg rafts were daily collected and placed in receptacles with dechlorinated water and then left undisturbed till hatching of larvae. After breeding of several generations, suitable specimens were selected for running the bioassay experiments.

Materials Used:

1. Plastic bottles, 1 L. capacity.
2. Dechlorinated water: Removing of chlorine from the water was achieved by filling a bucket with tap water and left uncovered at room temperature for 24 hours to allow evaporation of the chlorine.
3. Sucrose (white sugar).
4. Molasses obtained from the Egyptian Sugar and Integrated Industries Company in Hawamdiya, Giza, Egypt.
5. CAPL-star: prepared in Central Agricultural Pesticides Laboratory (CAPL*). It contains basically molasses as feeding material and ammonium carbonate as a source of ammonia emanation as attractive material.
6. Baker's yeast granules, *Saccharomyces cerevisiae*.
7. Lactic acid and Ammonium hydroxide 33% (Gamhouria Company for Trading Chemicals and Medical Supplies, Cairo, Egypt).

Testing Methods:

Attractive traps were formed according to Abdon-Liwanag and Tansengco (2015) and Sukumaran *et al.* (2015) with some modifications by using plastic soda bottles, 1 L. capacity which was cut in the half and the neck portions were set aside (Figure 1A) to be used after the solutions

(Table 1) were prepared, then the funnel part was placed upside down into the other half of the bottle and fixed with tape to make the cap hole the only way in-out for mosquitoes (Figure 1B). Each trap containing an attractant solution was wrapped by a dark silver paper then placed in a cage (30*30*30 cm) containing the tested mosquitoes (Schreck *et al.* 1990). Three trials (replicates) were made for each attractant solution. For each replicate, 25 of *Culex pipiens* females, aged (3-6) days old and fed only 10% sucrose were aspirated from the holding cages into paper cups using hand-held mouth aspirators, transferred into the test cage and kept at 27 ±2 °C and 70 ±10 % RH. After one day and two days, the number of attracted mosquitoes inside the trap was counted and the percentage for each treatment replicate was calculated as follow: Number of attracted mosquitoes in trap / number of total mosquitoes in the cage (25) x100.

Table 1: Components of the attracting solutions' tested against *Culex pipiens* females:

Materials	Tested Mixture
Sucrose (white sugar)	1- 50 gm sucrose + 100 ml warm water. 2- 50 gm sucrose + 100 ml warm water + 1 gm Baker's yeast granules.
Molasses	1- 50 gm molasses + 100 ml warm water. 2- 50 gm molasses + 100 ml warm water + 1 gm Baker's yeast granules.
CAPL*	1- 50 gm CAPL* + 100 ml warm water. 2- 50 gm CAPL* + 100 ml warm water + 1 gm Baker's yeast granules.
Ammonium hydroxide 33%	Different dilutions of ammonium hydroxide 33% dissolved in dechlorinated water and adjusted to 100 ml solution.
Lactic acid	Different dilutions of lactic acid dissolved in dechlorinated water and adjusted to 100 ml solution.
Lactic acid + molasses	5 µl lactic acid + 50 gm molasses + 100 ml dechlorinated water
Lactic acid + sucrose	5 µl lactic acid + 50 gm sucrose + 100 ml dechlorinated water

CAPL* contains molasses as feeding material and ammonium carbonate as attractive material.

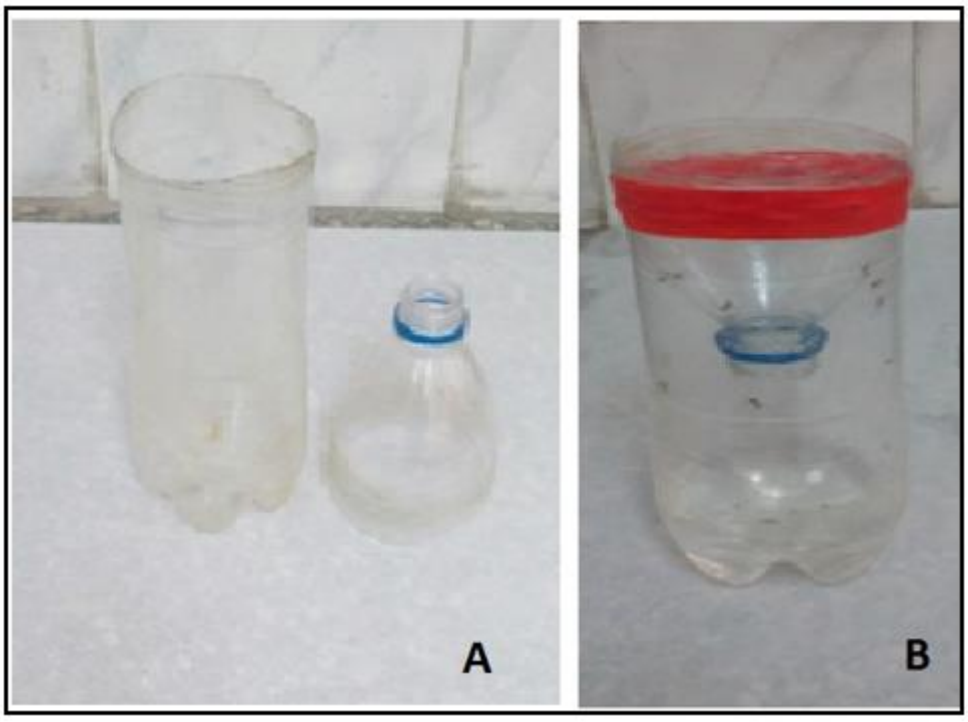

Fig 1: Attractive trap for mosquito adults.

Statistical Analysis:

Means and Standard Deviations (SD) were calculated for the different tests and compared by the one-way analysis of variance (ANOVA) and if significantly different, they were exposed to multiple comparisons by LSD (P = 0.05). The SAS computerized software (Anonymous 1998) was used for such analysis.

RESULTS AND DISCUSSION

Sugary Solutions:

In general, results in Table (2) indicate that all tested solutions gave different percentages of attraction. The attraction increased as the period of exposure increased and the addition of yeast to the solutions of sucrose, molasses, and CAPL* increased their attraction and caused death of all attracted mosquitoes.

CAPL* solution showed a higher attractive effect than sucrose solution or molasses solution as it contained beside molasses (food attractant) ammonium carbonate which considered a source of ammonia as attractive material. On the other hand, sucrose solution showed higher attractive effect than molasses solution and this agreed with Paturau (1982) who reported that molasses has low sucrose content, while this result disagreed with Mweresa *et al.* (2014) who reported that molasses is a suitable ingredient for the replacement of sucrose as a substrate for the production of CO_2 in presence of yeast for sampling of African malaria vectors and other mosquito species. Also, the attractive effect of sugary solutions increased by adding yeast due to emanation of CO_2 which is responsible for increased flight activity, attraction in some species and sensitization of mosquitoes to host odors (Gillies 1980; Dekker *et al.* 2005 and De Azara *et. al.* 2013).

Table 2: Effect of trap contents on the attraction of *Culex pipiens* females under laboratory conditions at 27±2 °C and 70±10% RH

Attractant	Effect	One day	Two days	Notes
		Mean % ± SD	Mean % ± SD	
50 gm sucrose + 100 ml warm water	Feeding	48.0±10.00 b	50.7±1.00 d	Alive attracted mosquitoes
50 gm sucrose + 1 gm Baker's yeast granules + 100 ml warm water	Feeding, Co_2 emanation	72.0±2.65 a	100.0±0.00 a	Dead attracted mosquitoes
50 gm molasses + 100 ml warm water	Feeding	44.0±1.00 b	46.7±0.58 d	Alive attracted mosquitoes
50 gm molasses + 1 gm Baker's yeast granules + 100 ml warm water	Feeding, Co_2 emanation	49.3±3.06 b	92.0±1.00 b	Dead attracted mosquitoes
50 gm CAPL* + 100 ml warm water	Feeding	69.3±2.89 a	72.0±1.73 c	Alive attracted mosquitoes
50 gm CAPL* + 1 gm Baker's yeast granules + 100 ml warm water	Feeding, Co_2 emanation NH_3 emanation	74.7±0.58 a	100.0±0.00 a	Dead attracted mosquitoes
F- value		7.99	129.40	
P		0.0016	<0.0001	

CAPL* contains molasses as feeding material and ammonium carbonate as attractive material.
Vertically, means with the same letters are not significantly different, LSD (P > 0.05).

Mixing sugary solutions with 1gm Baker's yeast granules to release CO_2 not only increased the attraction of mosquito females but also caused death to all attracted mosquitoes inside trap which is agreed with Iked *et al.* (1989). Using each of sucrose solution, molasses solution and CAPL* solution alone attracted 50.7%, 46.7%, and 72.0%, after two days exposure, respectively while in case of mixing yeast with sucrose, molasses and CAPL*, gave 100%, 92%, and 100% attraction after two days exposure respectively. These results agreed with Jerry *et al.* (2017) report that the solution containing high amount of sugar produced a large volume of CO_2 for longest time period and sufficient amount of yeast significantly increased CO_2 production from the sugar mixture and also agreed with other observations (Saitoh *et al.*, 2004; Smallegange *et al.*, 2010 and Abdon-Liwanag and Tansengco 2015).

Moreover, the obtained results show that the highest performed solution whether after one day or two days was CAPL* solution mixed with Baker's yeast granules (74.7% and 100% attraction after day one and day two, respectively). This was followed by sucrose solution mixed with Baker's yeast granules, then molasses solution with Baker's yeast granules, so that we recommend using these solutions according to their attraction effect.

Table 3: Effect of different dilutions of ammonium hydroxide (33%) on the attraction of *Culex pipiens* females under laboratory conditions at27±2 °C and 70±10% RH

Attractant	Effect	One day	Two days	Notes
		Mean % ± SD	Mean % ± SD	
100 ml NH4OH	NH_3 emanation	0.0±0.00 f	2.7±0.58 i	alive attracted mosquitoes
50 ml NH4OH +50 ml dechlorinated water		0.0±0.00 f	4.0±0.00 h	
10 ml NH4OH + 90 ml dechlorinated water		12.0±0.00 e	28.0±0.00 g	
5 ml NH4OH + 95 ml dechlorinated water		21.3±0.58 d	28.0±0.00 g	
1 ml NH4OH + 99 ml dechlorinated water		32.0±1.00 c	33.3±0.58 fg	
0.5 ml NH4OH + 99.5 ml dechlorinated water		32.0±0.00 c	36.0±0.00 ef	
100 µl NH4OH + 100 ml dechlorinated water		36.0±0.00 bc	40.0±0.00 e	
50 µl NH4OH + 100 ml dechlorinated water		36.0±0.00 bc	48.0±0.00 d	
10 µl NH4OH + 100 ml dechlorinated water		40.0±1.00 bc	49.3±0.58 d	
5 µl NH4OH + 100 ml dechlorinated water		40.0±1.00 bc	70.7±0.58 c	
1 µl NH4OH + 100 ml dechlorinated water		77.3±0.58 a	100.0±0.00 a	
0.5 µl NH4OH + 100 ml dechlorinated water		80.0±1.00 a	84.0±1.00 b	
F- value		308.49	301.22	
P		<.0001	<.0001	
At Day 2: percent values were transferred to Arcsine before conducting analysis of variance				

Vertically, means with the same letters are not significantly different, LSD (P > 0.05)

Ammonium Hydroxide (33%) Solutions:

The obtained results (Table 3) indicate that the attraction effect of ammonium hydroxide which considered a source of ammonia as an attractive material increased as its concentration decreased up to 1 µl/100 ml water which gave 77.3% and 100% attraction after one day and two days exposure, respectively. On the other hand, the attraction percentage after two days of exposure decreased as the concentration decreased than 1 µl/100 ml water, since 0.5 µl/100 ml water gave 84% mosquito attraction . These results agreed with that obtained by Geier *et al.* (2000) and Braks *et al.* (2001).

Lactic Acid Solutions:

Examining the efficacy of Lactic acid to attract adult females of *Culex pipiens* mosquitoes (Table 4) revealed that the different water dilutions of lactic acid gave lower attraction to mosquito females in comparison to ammonium hydroxide (33%) where 5 µl lactic acid /100 ml water which showed the highest attraction gave only 58.7% and 60% attraction after one day and two days, respectively. It was found that adding molasses or sucrose to the 5 µl lactic acid /100 ml water improved the attraction, which resulted in 92% and 96% attraction of mosquitoes after one day and two days, respectively by adding molasses, while, by adding sucrose, the attraction percentages were 76% and 92% at first day and second day, respectively. These data indicate that lactic acid alone is not suitable to be used as attractive material but adding of molasses or sucrose may improve lactic acid attraction in agreement with the previous studies (Bernier *et al*., 2000 & 2007; Geier *et al.,* 2000; Braks *et al.,* 2001 and Dekker *et al.,* 2002).

It was reported that blood-seeking mosquitoes use different olfactory cues to locate a host (Takken 1991) of which ammonia and lactic acid are the constituents of sweat and skin emanations (Cork and Park, 1996; Geier *et al.,* 1996 and Barks *et al.,* 2001).

Mode of Action of the Tested Materials:

The attractive effect of sugary solutions (sucrose, molasses, and CAPL*) is due to their food attractive effect (Ohm, 2018) and that of CAPL* which showed higher attraction efficacy than the other sugary solutions is due to releasing of ammonia which is considered as an attractive material (Geier *et al.,* 2000 and Braks *et al.,* 2001) since it contains ammonium carbonate.

Mixing of Baker's yeast granules with sugary solutions caused increasing of attractive effect due to releasing of carbon dioxide which is considered as an attractant to mosquito adult females (Saitoh *et al.,* 2004; Smallegange *et al.,* 2010 and Abdon-Liwanag and Tansengco, 2015), Moreover, death of the attracted mosquito females in case of mixing yeast with sugary solutions is due to releasing of CO_2 which has both asphyxiate and poisoning effect (Ikeda *et al.,* 1989 and Abdon-Liwanag and Tansengco, 2015). The attractive effect of ammonium hydroxide is due to releasing of ammonia (Geier *et al.,* 2000 and Braks *et al.,* 2001). Lactic acid is considered a weak attractive material (Bernier *et al*., 2000 & 2007; Geier *et al.,* 2000; Braks *et al.,* 2001 and Dekker *et al.,* 2002).

Table 4: Effect of different dilutions of lactic acid on the attraction of *Culex pipiens* females under laboratory conditions at 27±2 °C and 70±10% RH

Attractant	Effect	One day	Two days	Notes
		Mean % ± SD	Mean % ± SD	
100 ml lactic acid	Lactic acid emanation	20.0±0.00 i	24.0±1.00 i	alive attracted mosquitoes
50 ml lactic acid + 50 ml dechlorinated water		28.0±0.00 h	29.3±0.58 hi	
10 ml lactic acid + 90 ml dechlorinated water		32.0±1.00 gh	33.3±0.58 ghi	
5 ml lactic acid + 95 ml dechlorinated water		32.0±0.00 gh	36.0±1.00 fgh	
1 ml lactic acid + 99 ml dechlorinated water		32.0±1.73 gh	40.0±1.00 efg	
0.5 ml lactic acid + 99.5 ml dechlorinated water		36.0±0.00 fg	40.0±0.00 efg	
100 µl lactic acid + 100 ml dechlorinated water		40.0±1.00 ef	42.7±0.58 efg	
50 µl lactic acid + 100 ml dechlorinated water		44.0±0.00 ef	48.0±1.00 de	
10 µl lactic acid + 100 ml dechlorinated water		52.0±2.00 d	54.7±0.58 cd	
5 µl lactic acid + 100 ml dechlorinated water		58.7±0.58 c	60.0±1.00 cd	
1 µl lactic acid + 100 ml dechlorinated water		42.7±0.58 ef	44.0±1.73 def	
5 µl lactic acid + 50 gm molasses+ 100 ml dechlorinated water	Feeding, lactic acid emanation	92.0±0.00 a	96.0±1.00 a	
5 µl lactic acid + 50 gm white sugar+ 100 ml dechlorinated water	Feeding, lactic acid emanation	76.0±1.73 b	92.0±0.00 b	
F value		63.13	61.76	
P		<.0001	<.0001	
At Day 2 percent values were transferred to Arcsine before conducting analysis of variance				

Vertically, means with the same letters are not significantly different, LSD (P > 0.05)

CONCLUSION

From the discussion, it can be concluded that, the material which gave ≥ 90% attraction after two days of exposure at laboratory conditions solutions of sucrose + yeast, molasses + yeast, CAPL* + yeast, ammonium hydroxide (33%) at 1 µl/100 ml water and lactic acid at 5 µl/100 ml mixed with sucrose or molasses are considered successful as attractants to adult females of *Culex pipiens* mosquitoes. Moreover, these solutions showed 100% mortality of the attracted mosquitoes, so that they may be regarded as mosquito control agents. However, further intensive studies are needed to evaluate their efficacy as mosquito control agents under field conditions.

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ARABIC SUMMARY

تقييم معملى لبعض المركبات المحليه كجاذبات لاناث بعوضة كيولكس بيبانز

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أجريت تجارب معملية لتقييم فعالية بعض المركبات الآمنة بيئياً لجذب إناث البعوض البالغ *Culex pipiens* حيث أوضحت النتائج أن ثاني أكسيد الكربون له تأثير جاذب قوي في محاليل السكروز وكابل ستار والمولاس بإضافة الخميرة إلى كل منها حيث أدى ذلك إلى انجذاب 100%, 100%, 92% من إناث البعوض البالغة بعد يومين من التعرض على لتوالي. كما أوضحت النتائج إن انبعاث ثاني أكسيد الكربون يؤدي إلى موت إناث البعوض المنجذبة. كما أوضحت النتائج أن هيدروكسيد الأمونيوم (33%) يعتبر جاذب قوي لإناث بعوضة الكيولكس بيبينز باستخدام جرعات منخفضة بينما يعتبر حمض اللاكتيك جاذب ضعيف لإناث بعوضة الكيولكس بيبينز ولكن بإضافة السكروز والعسل الأسود كل على حده أدى ذلك إلى زيادة جذب إناث بعوضة الكيولكس.