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Yeast-generated CO_2: A convenient source of carbon dioxide for mosquito trapping using the BG-Sentinel® traps

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ABSTRACT

Objectives: To evaluates carbon dioxide (CO_2) production from yeast/sugar mixtures and its efficiency as an attractant in BG-Sentinel traps.

Methods: The rate of CO_2 production was optimized for different yeast/sugar mixtures. The optimized mixture was then used as bait in BG-Sentinel traps. The efficiency of this bait was then compared to octenol baited traps.

Results: The yeast/sugar (5 g: 280 g) in 300 mL water generated the highest volume of CO_2. The CO_2 baited traps caught significantly more mosquitoes than octenol baited traps.

Conclusions: Yeast-produced CO_2 can effectively replace octenol baits in BG traps. This will significantly reduce costs and allow sustainable mass-application of the CO_2 baited traps in large scale surveillance programs.

1. Introduction

Chemical cues such as carbon dioxide (CO_2) are important for the host-finding behaviour of mosquitoes [1–4]. Surveillance programs which utilize mosquito trapping often include chemical baits such as CO_2, octenol, nonanol or lactic acid to increase catch rates [5–11]. In Trinidad and Tobago, surveillance and/or sampling exercises utilise the BG-Sentinel® trap [12] baited with either octenol or dry ice which generates CO_2. However, one of the limiting factors is the cost and availability of octenol and dry ice [13,14]. The BG-Sentinel® trap is a well-established monitoring tool for capturing mosquitoes [15], however, the effectiveness of yeast/sugar generated CO_2 bait has not been evaluated for use in Trinidad and Tobago.

Various studies have previously reported on the efficacy of octenol and carbon dioxide (CO_2) as attractants in mosquito traps such as the Fay-Prince trap, CDC-type and the Encephalitis Virus Surveillance traps [15–24]. These studies suggest that octenol may have species-specific effects. Kleine *et al.* [16] also reported that octenol differs in its effectiveness for attracting different mosquito species. However, Octenol does not appear to be a strong attractant for Stegomyia mosquitoes which includes *Aedes aegypti* (*Ae. aegypti*), an important vector for the spread of tropical diseases such as dengue, zika and chikungunya, which has a high prevalence rate in the Caribbean region. Canyon and Hii [6] reported that octenol significantly decrease collection of *Ae. aegypti* when compared to carbon dioxide using Fay-Prince traps. Shone *et al.* [25] reported that species such as *Aedes albopictus* was attracted more to CO_2 and CO_2+ octanol baited CDC and Fay-Prince traps than unbaited or octenol-baited traps. These studies all suggest that the choice of bait can effectively increase the catch efficiency of a mosquito trap.

In Trinidad and Tobago, the preferred bait is mosquito traps is octanol, though dry ice is sometimes used. However this is neither cost effective nor sustainable when traps are deployed in remote areas or when large scale sampling is needed, as is the case when there is an upsurge in the number of cases of dengue, zika and chikungunya. In tropical environments, dry ice sublimes faster than in temperate areas and has to be replaced frequently. Moreover, dry ice has the disadvantage that the release rate of CO_2 is highly variable and diminishes over time [26,27]. When large scale trapping is required, such as during a national surveillance program, the use of both dry ice and octenol can become prohibitively expensive. To overcome these limitations, CO_2 produced by fermentation of sugar can

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be a reliable alternate that is cheap, easy to manage and durable. This paper seeks to optimize CO_2 production from fermentation of sugar and compare its efficiency to octenol for capturing mosquito in baited BG-sentinel traps.

2. Materials and methods

2.1. Carbon dioxide production from yeast/sugar mixture

Three hundred (300) mL of 130, 190, 250 and 280 g/L sugar solutions each containing 3 g of baker's yeast was prepared in 500 mL Buchner flasks and maintained at 35 °C in a water bath. The rate of gas production was determined using a displacement method. The buchner flask was connected to a sealed conical flask filled with water and fitted with a displacement tube which emptied into a graduated cylinder. The volume of fluid displaced per unit time was used to calculate CO_2 production rates over 24 h.

2.2. Optimization of yeast for carbon dioxide production

Four reaction flasks containing 280 g/L sugar solution were prepared to assess the effects of yeast on the CO_2 production. Aliquot 1.5 g and 5 g of yeast was added to duplicate flask, and the rate of gas production was monitored over 24 h. Estimation of the carbon dioxide production was determined as described previously.

2.3. Field evaluation of yeast-generated carbon dioxide in mosquito collections

The optimized reaction mixture was then tested in field trials using the BG trap. Carbon dioxide baited traps were run simultaneously with octenol baited traps to compare the catch efficiency of the two baits. A total of 45 sampling efforts were conducted from January to May 2017 at two sample locations: (1) Open green house and (2) in dwellings. The total number of mosquitoes was counted and the number of species compared.

3. Results

The volume of carbon dioxide generated from the sugar solution with 3 g of yeast generally increased over the first (4–6) h then gradually decreased (Figure 1). Production rate after 1 h ranged between 3.1 mL/min (130 g/L solution) and 6.2 mL/min (280 g/L solution) (Figure 1). The 280 g/L solution generated significantly ($P < 0.05$) higher levels of CO_2 when compared to the 130 g/L solution. However CO_2 production levels in 280 g/L solution were not significantly higher than production from 190 g/L and 250 g/L solutions. The total volume of CO_2 generated varied between 3 L (130 g/L solution) and 5 L (280 g/L solution) over the first 7 h. After 24 h, CO_2 production rates significantly decreased, ranging between 0.73 mL/min (130 g/L solution) and 4.4 mL/min (280 g/L solution). However, over the 24 h period all the mixtures were still generation CO_2 at levels that were higher than the estimate CO_2 release rate of (1–1.8) mL/h from human skin [28]. This would suggest that the system would continue to attract mosquitoes over an extended period of time.

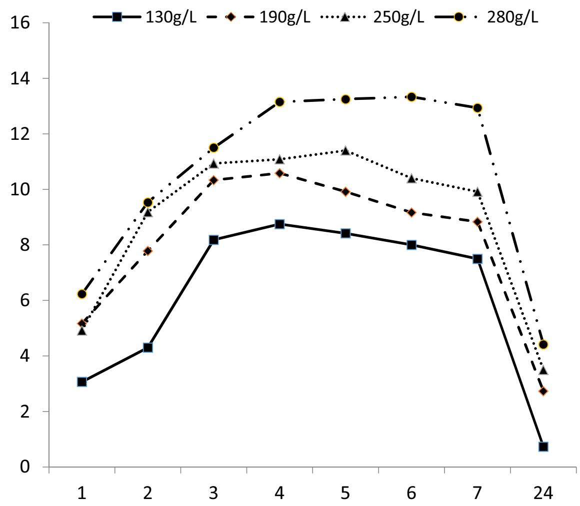

Figure 1. CO_2 production rated using different masses of sugar.

3.1. Optimization of yeast for carbon dioxide production

The amount of carbon dioxide generated can be influenced by the rate of fermentation and the amount of yeast added. From the first experiment, the solution containing 280 g of sugar produced the largest volume of CO_2 for the longest time period. Varying yeast concentration (1.5 g, 3.0 g and 5.0 g) significantly increased CO_2 production from the sugar mixture (Figure 2). Production rate after 1 h ranged between 3 mL/min (1.5 g yeast and 280 g/L sugar solution) and 10 mL/min (5 g yeast and 280 g/L sugar solution) (Figure 2). The solution containing 5 g of yeast produced significantly ($P < 0.05$) more CO_2 than the one containing 1.5 g of yeast (Figure 2). After 7 h CO_2 production rate ranged between 6.8 mL/min (1.5 g yeast and 280 g/L sugar solution) and 14.1 mL/min (5 g yeast and 280 g/L sugar solution) (Figure 2). After 24 h CO_2 production rates was still high, ranging between 5.9 mL/min (1.5 g yeast and 280 g/L sugar solution) and 5.5 mL/min (5 g yeast and 280 g/L sugar solution) (Figure 2). The highest level of CO_2 was produced after 3 h averaging 8.8 mL/min (1.5 g yeast) and 21.7 mL/min for the mixture with 5 g of yeast.

The total volume of CO_2 generated varied between 3 L (1.5 g yeast in 280 g/L sugar solution) and 7 L (5 g yeast in 280 g/L sugar solution) within the first 7 h. After 24 h production rates were significantly reduced ranging between 6 mL/min (1.5 g yeast in 280 g/L sugar solution) and 4.4 mL/min (3 g in 280 g/L sugar solution). However over the 24 h period all the reaction mixtures were still generating sufficiently high levels of CO_2.

3.2. Field evaluation of yeast-generated carbon dioxide, in mosquito collections

A total of 45 field trials (30 open green house and 15 in dwellings) were conducted using both CO_2 baited and octenol baited BG traps. *Culex quinquefasciatus* (*Cx. quinquefasciatus*) was the dominant species caught at both sites. A total of 842 mosquitoes were collected which consisted primarily of *Cx. quinquefasciatus* (84.1%) and *Ae. aegypti* (15.9%). The total number of mosquitoes collected with the CO_2 baited BG traps (620 mosquitoes) was three times higher than the number collected with the octenol baited (222 mosquitoes) traps. This

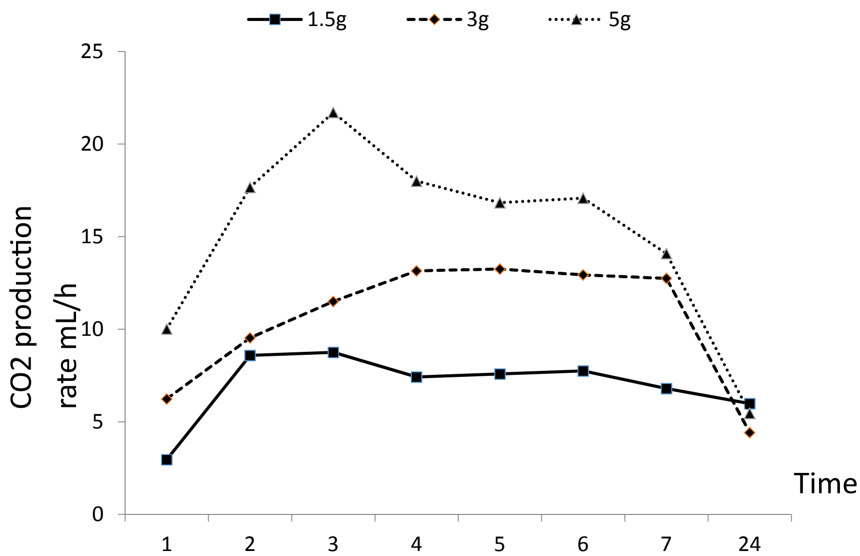

Figure 2. CO_2 production rated from varying masses of yeast.

would suggest that the catch efficiency of CO_2 baited traps was greater than the octenol bait.

The CO_2 baited traps attracted about 4 times more *Cx. quinquefasciatus* (555) than octenol (153) baited traps. However both baits attracted similar numbers of *Ae. aegypti*. In the open green house, the CO_2 baited traps collected twice as much *Cx. quinquefasciatus* (216) than the octenol (112) baited traps. The CO_2 bait traps collected 20% less *Ae. aegypti* (40) when compared to the octenol baited (60). At the site within the dwellings, the CO_2 baited collected eleven times more *Cx. quinquefasciatus* (339) when compared to the octanol baited traps (41). The CO_2 baited traps also collected three times more *Ae. aegypti* (25) than the octenol baited traps (9). The octenol baited traps had 42% males and 58% females while the CO_2 baited trap had 52% males and 48% females. However, all of *Cx. quinquefasciatus* collected were females.

4. Discussion

Mosquitoes respond to a complex set of cues such as carbon dioxide, lactic acid or temperature to locate a host. In Trinidad and Tobago, surveillance programs utilize the BG sentinel traps, which have been shown to be more effective in capturing *Aedes* sp. than other traps such as the CDC-LT [8,29]. The BG traps are normally baited with octenol, however, the cost can be prohibitive for large scale monitoring. This study showed that carbon dioxide generated from yeast/sugar mixtures can be a more efficient attractant than octenol in BG traps. The optimum mixture used in this study, which produced the highest amount CO_2 was 5 g yeast and 280 g/L sugar solution.

Various studies previously reported on the catch efficiency of different attractants. Several studies have also reported that few species were attracted to octenol alone, and catch rates increases when it is used in combination with other attractants [22,23,30]. Kline *et al*. [31–34] also reported that different mosquito species sometimes respond differently to different attractants. The basic response pattern was that very few species were attracted to octenol alone, but in combination with CO_2 a synergistic affects apparently occurred and catch efficiency increases 2-fold or greater. This present study showed that CO_2 was about 3 times more efficient at capturing mosquitoes than octenol alone using the BG Traps. However, previous studies on African and Brazilian malaria vectors, such as *Anopheles arabiensis*, *Anopheles funestus*, *Anopheles darlingi* and *Anopheles aquasalis* have shown that CO_2 was insufficiently attractive as standalone bait. Better catch rates were obtained using CO_2 in mixed odour baits or together with body odours [35–37].

The BG traps though especially developed for capturing *Ae. aegypti*, have also been shown to capture *Culex* mosquitoes [12,38]. This present study showed that the BG traps baited with CO_2 had a catch rate about 8 times higher for *Cx. quinquefasciatus* than *Ae. aegypti*. Other studies have also reported that BG traps baited with CO_2 do have high catch efficiencies [39–41]. Ferreira de Ázara *et al*. [42] also reported that BG traps operated with CO_2 trapped 6 times more female *Culex* spp than *Ae. aegypti*. The high catch rates of up to 272 *Culex* females with CO_2 and up to 57 *Culex* females without CO_2 (mainly *Cx. quinquefasciatus*) per 24 h shows that the BGs trap might be a useful tool for the monitoring of diseases that are transmitted by the species in urban areas in Brazil, like Oropouche fever or Bancroftian Filariosis. This study further emphasises that CO_2 attracted only female Culex, while it attracted both male and female aedes. This would suggest that CO_2 is also an attractant for *Culex* species which may be due to the BG's trap imitation of human odour plumes. Russell [43] also reported improved collection of *Cx. quinquefasciatus* in CO_2 baited traps for in French Polynesia while Muturi *et al*. [44] reported high levels if *Cx. quinquefasciatus* and *Culex annulioris* in Kenya. Zhang *et al*. [45] also showed that CDC-LT with dry ice was most effective for trapping of *Cx. quinquefasciatus* when compared with UV light traps and gravid traps in China. Smallegange *et al*. [46] also showed that traps baited with yeast-produced CO_2 caught significantly more mosquitoes than unbaited traps and traps baited with industrial CO_2. They suggested that yeast-produced CO_2 can effectively replace industrial CO_2 for sampling of mosquitoes such as *Anopheles gambiae*. The use of the yeast/sugar generated CO_2 would significantly reduce costs and allow sustainable mass-application of traps for mosquito sampling in remote areas.

Given the recent upsurgence of vector borne diseases such as dengue, chikungunya, and zika, greater efforts are being made to control mosquito populations with an increased in surveillance efforts. The success of the yeast/sugar mixture as bait in the BG traps can greatly reduce the cost of surveillance and increase the efficiency of mosquito capture.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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