

## Research Article

# Interactions among Carbon Dioxide, Heat, and Chemical Lures in Attracting the Bed Bug, *Cimex lectularius* L. (Hemiptera: Cimicidae)

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Commercial bed bug (*Cimex lectularius* L.) monitors incorporating carbon dioxide (CO<sub>2</sub>), heat, and chemical lures are being used for detecting bed bugs; however, there are few reported studies on the effectiveness of chemical lures in bed bug monitors and the interactions among chemical lure, CO<sub>2</sub>, and heat. We screened 12 chemicals for their attraction to bed bugs and evaluated interactions among chemical lures, CO<sub>2</sub>, and heat. The chemical lure mixture consisting of nonanal, 1-octen-3-ol, spearmint oil, and coriander Egyptian oil was found to be most attractive to bed bugs and significantly increased the trap catches in laboratory bioassays. Adding this chemical lure mixture when CO<sub>2</sub> was present increased the trap catches compared with traps baited with CO<sub>2</sub> alone, whereas adding heat did not significantly increase trap catches when CO<sub>2</sub> was present. Results suggest a combination of chemical lure and CO<sub>2</sub> is essential for designing effective bed bug monitors.

## 1. Introduction

Hematophagous arthropods use a variety of visual, mechanical, chemical, and thermal cues to detect vertebrate hosts [1]. Host searching behavior in unfed bont tick, *Amblyomma hebraeum* Koch [2, 3], and *Glossina* spp. (Diptera: Glossinidae) [4] is stimulated by carbon dioxide (CO<sub>2</sub>) emitted by mammalian hosts. Odors from human skin [5], sweat, breath and body odors from cattle, birds, and mice [6], bird feathers or skin [7], and bird uropygial glands [8] play a major role in attracting different families of hematophagous mosquitoes. R-(–)-1-octen-3-ol, an enantiomer of 1-octen-3-ol, was found attractive to field populations of adult mosquitoes [9]. Geranyl acetone (E and Z enantiomers), a component of human sweat, elicited strong electroantennogram responses in female *Anopheles gambiae* Giles [10].

The resurgence of bed bugs (*Cimex lectularius* L.) in recent years stimulated research on bed bug behavior [11, 12] with the goal of developing effective bed bug monitoring

tools. It is known that bed bugs use CO<sub>2</sub> [11–13], heat, and chemical odors to locate their hosts [11, 12, 14, 15]. Among the chemical lures, geranyl acetone, 1-octen-3-ol, and L-lactic acid have been reported to be attractive to bed bugs [16, 17]. Bed bug airborne aggregation pheromones including (E)-2-hexenal, (E)-2-octenal, (2E, 4E)-octadienal, benzaldehyde, nonanal, decanal, sulcatone, (+)-limonene, (–)-limonene, and benzyl alcohol were attractive to bed bug nymphs in olfactometer bioassays [18]. These chemicals could potentially be used for monitoring bed bugs; however their effectiveness has not been tested yet in arenas or under conditions that simulate field conditions.

Anderson et al. [11] demonstrated the effectiveness of a trap baited with CO<sub>2</sub> (50–400 mL/min), heat (37.2–42.2°C), and a chemical lure comprised of 33.0 µg propionic acid, 0.33 µg butyric acid, 0.33 µg valeric acid, 100 µg 1-octen-3-ol (octenol), and 100 µg L-lactic acid. In a separate study, Wang et al. [12] confirmed the effectiveness of CO<sub>2</sub> (169 mL/min) and heat (43.3–48.8°C) in their attraction to bed bugs. Until

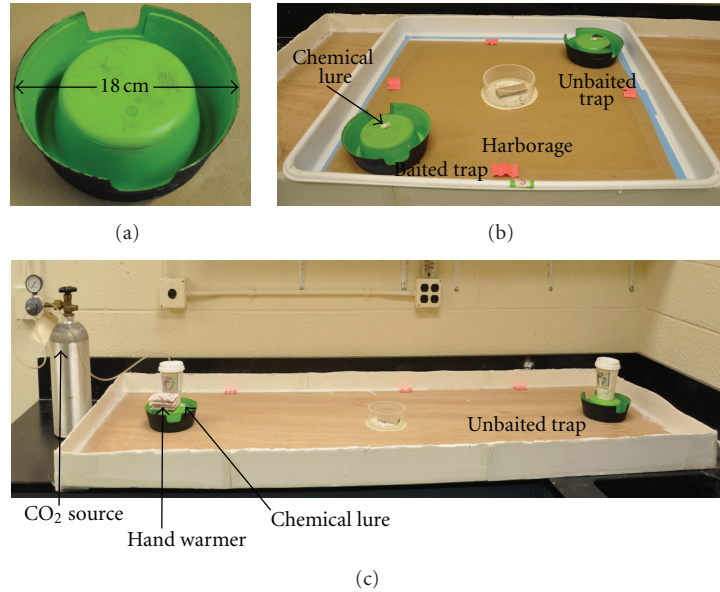


FIGURE 1: Experimental setup for determining bed bug attraction to nonchemical and chemical lures: (a) pitfall trap used in all bioassays; (b) a plastic tray arena with a baited and an unbaited trap; (c) a wooden door arena with a baited trap and an unbaited trap.

present, there are no studies investigating the interactions among chemical lures, heat, and CO<sub>2</sub>.

Bed bugs hide during the day and are difficult to locate as they are small and elusive. Therefore, developing effective monitoring tools has been recognized as a critical component in the current campaign for fighting the bed bug resurgence [19]. Most of the available monitors incorporate one or several nonchemical and chemical lures to attract and capture hungry bed bugs foraging for blood meals. However, the data on the role of various lures in the effectiveness of monitors are very limited. Studying the interactions among nonchemical and chemical lures has immediate practical significance in designing more effective monitors which can be used to detect the presence of small numbers of bed bugs or as an alternative control method. The objectives of this study were (1) screening for chemical lures that are attractive to bed bugs, (2) testing the effect of CO<sub>2</sub> release rate and heat source on trap catches and (3) determining the interactions among chemical lures, CO<sub>2</sub>, and heat in attracting bed bugs.

## 2. Material and Methods

**2.1. Insects.** Bed bugs were collected from an infested house in Lakewood, NJ. They were maintained in plastic containers (4.7 cm height and 5 cm diameter) with folded paper as harborages at 26°C ± 1°C, 40 ± 10% relative humidity, a 12:12-hour (L:D) photoperiod, and were deprived of food for the entire duration of the study. There was a great variation in their hunger levels ranging from very hungry to very well fed at the time of collection. We immediately started the experiments after collection using hungry bugs based on color of the insect abdomen. Only males and large bed bug nymphs were used in this study. Females were not tested to avoid mating and laying eggs in the arenas. All bioassays were conducted within 3 months after bed bugs were collected.

**2.2. Pitfall Trap and Experimental Arenas.** Pitfall traps were used to evaluate the attractiveness of various lures. The pitfall trap was an inverted plastic dog bowl (600 mL volume, 18 cm diameter, 6.4 cm depth, and 1 mm thickness) (IKEA, Baltimore, MD, USA) (Figure 1(a)). The outer wall of the trap was covered with a layer of paper surgical tape (Caring International, Mundelein, IL, USA), which was painted black with ColorPlace spray paint (WalMart Stores Inc., Bentonville, USA). Bed bugs preferred black color to white color in our preliminary bioassays.

Two types of experimental arenas were used: (a) wooden door arenas (200 by 76 cm by 6.4 cm) (length by width by height) with wooden floor and (b) plastic tray arenas (80 by 75 by 5 cm) (length by width by height) with bottom lined with brown paper (Figure 1(b)). The brown paper was never changed during the entire study. A layer of fluoropolymer resin (DuPont Polymers, Wilmington, DE, USA) was applied to inner walls of the experimental arenas to prevent the bugs from escaping. A layer of this resin was also applied to inner walls of the pitfall traps in a similar fashion to confine the bed bugs that fell into the traps. A filter paper (15 cm diameter) was placed on the floor in the center of each arena, and then a plastic ring (13.3 cm diameter and 6.4 cm height) was placed on the filter paper for confining the bed bugs. A piece of folded cardboard and folded fabric was placed on the filter paper inside the ring to provide harborages for bed bugs. Six and four additional paper harborages measuring 5.1 cm long and 3.3 cm wide were placed along the edges of the floor of each wooden and tray arena, respectively. Two wooden door arenas were located at least 6 m away from each other in a 15 m long and 9 m wide room at 23–25°C. Two additional wooden door arenas were located in two 4 m long and 2.3 m wide rooms at 24–25°C. These rooms had air current through vents on the ceilings or through the open door. In experiments using plastic tray arenas, four arenas

were placed simultaneously in a nonventilated, closed room measuring 4 m long and 2.3 m wide at 24–25°C. A 12:12-hour (L:D) cycle was maintained in all the rooms that were used for bioassays.

**2.3. Effect of CO<sub>2</sub> Release Rate on Bed Bug Trap Efficacy.** Four door arenas were used and each arena had an unbaited control pitfall trap and a pitfall trap baited with CO<sub>2</sub>. The two traps were placed at opposite ends equidistant (85 cm) from the center. The experiment was tested over 4 consecutive days. On each day, a different CO<sub>2</sub> release rate was used in each arena following a Latin square design. The CO<sub>2</sub> source was 5 lb cylinders (Airgas East Inc., Piscataway, NJ, USA). The tested release rates were 200, 300, 400, and 500 mL/min. The rate was determined as mL of bubble fluid displaced by CO<sub>2</sub> per unit of time using a Bubble-O-Meter (Bubble-O-Meter, Dublin, Ohio, USA). The CO<sub>2</sub> was introduced into 240 mL plastic cups that were placed on the pitfall traps (Figure 1(c)). Two holes were made on the lid of each plastic cup for CO<sub>2</sub> to escape. Fifty bed bug nymphs and adult males were released into the center of each arena and confined with a plastic ring. The bugs were acclimated for approximately 15 hours prior to the start of the experiment. At 1 hour after dark cycle, CO<sub>2</sub> was released and the plastic ring confining the bugs was removed. The numbers of bed bugs trapped in the pitfall traps and those in the arenas were collected and counted only after 8 hours with the aid of a flashlight. An 8-hour period has been observed to be sufficient for observing the effect of lures on bed bug behavior in preliminary bioassays. After counting, dead and moribund bugs were replaced with healthy bugs in each arena. All bugs were placed back to the center of the arenas and confined with plastic rings for 15 hours before starting the next bioassay.

**2.4. Effect of Heat on Bed Bug Trap Efficacy.** This experiment was conducted in four plastic tray arenas. Mini hand warmers were used as the heat source (Grabber, Grand Rapids, MI, USA). Two pitfall traps were placed at opposite corners of each arena equidistant (25 cm) from the center. One trap received either two or four mini hand warmers, and the other trap was used as an unbaited control. The surface temperature of the hand warmer was 40–48°C during the first 6 hours. The air temperatures on the floor of arenas 1 cm away from the pitfall trap baited with 2 and 4 hand warmers were 0.2–0.3°C and 0.5–0.6°C, above the ambient temperature, respectively. The air temperatures at the lip of pitfall trap baited with 2 and 4 hand warmers were 0.8–0.9°C and 1.3–1.6°C, above the ambient temperature, respectively. These temperatures were based on hourly recordings of one monitor during the first 6 hours after trap placement using a thermocouple thermometer (Cole-Parmer Instrument Company, Vernon Hills, IL, USA). The ambient temperature was recorded in the center of each arena equidistant (25 cm) from all traps and 3 cm above arena floor. Each treatment was replicated 6 times over 3 consecutive days. Fifty bed bugs were released into each arena and the testing procedure was the same as that in Section 2.3.

**2.5. Effect of Heat on Bed Bug Trap Efficacy When CO<sub>2</sub> is Present.** CO<sub>2</sub> at 200 mL/min was selected based on results from Section 2.3. This rate is similar to the respiration rate of an adult human at rest (250 mL/min) [20]. CO<sub>2</sub> alone or in combination with 2, 3, or 4 mini hand warmers was tested in four wooden door arenas on the same day under similar conditions to those in Section 2.3. Each treatment was assigned to a different arena, and the experiment was repeated four times over four consecutive days following a Latin square design. Each arena had an unbaited control trap and a baited pitfall trap placed on opposite ends of the test arena. Fifty bed bugs were released into each arena and the testing procedure was the same as that in Section 2.3.

**2.6. Screening of Chemical Lures for Attraction to Bed Bugs in Four-Choice Bioassays.** Twelve known or potential bed bug chemical lures (Table 1) were evaluated for their attractiveness to bed bugs in plastic tray arenas. Most of them were provided by Bedoukian Research Inc. (Danbury, CT, USA). Three chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). One chemical was purchased from New Directions Aromatic (Ontario, Canada). Among them, styralol, benzyl alcohol, 6-methyl-5-hepten-2-one, and Insect Biting Lure, were potentially attractive to bed bugs (Robert Bedoukian, personal communication). The chemicals were randomly divided into 4 groups. Each group was tested in the same arenas to evaluate the attractiveness of the chemicals. A 50 µL aliquot of each chemical was dispensed on cotton within a 0.7 mL microcentrifuge tube. The lid of each tube had a 2 mm diameter opening to allow for slow release of the chemical. Four pitfall traps were placed at four corners equidistant (25 cm) from the center. Three traps in each arena were baited with three different chemical lures belonging to the same group listed in Table 1 and the fourth trap was an unbaited control. Each group of chemical lures was tested 8 times over two consecutive days. Fifty bed bugs were released into each arena and the testing procedure was the same as that in Section 2.3.

**2.7. Attractiveness of Chemical Lures to Bed Bugs in Two-Choice Bioassays.** Nonanal, 1-octen-3-ol, spearmint oil, coriander Egyptian oil, L-lactic acid, and L-carvone exhibited significant attraction to bed bugs in Section 2.6. These chemicals were further evaluated to confirm their attractiveness to bed bugs using two-choice bioassays. The experimental setup and testing procedure were similar to Section 2.6. The difference was that only two traps were placed at opposite corners of each arena (Figure 1(b)). One trap was used as an unbaited control and the other trap received a chemical lure. Each chemical lure was evaluated 8 times over two consecutive days. The baited and unbaited trap positions in each arena were switched on the second day to eliminate any positional effect that could influence the trap catch.

**2.8. Relative Attractiveness of Chemical Lures to Bed Bugs in Four-Choice Bioassays.** The relative attractiveness of four most effective chemicals, nonanal, 1-octen-3-ol, spearmint oil, and coriander Egyptian oil identified from Section 2.7,

TABLE 1: Percent bed bugs in pitfall traps baited with three chemical lures and an unbaited control in each arena.

Group	Chemical lure	<i>N</i>	Mean (%) $\pm$ SE	<i>F</i>	<i>P</i> value	Source of material
I	1-Octen-3-ol	8	28.3 $\pm$ 2.5*	8.60	0.0001	Bedoukian Research Inc.
	L-Lactic acid	8	25.7 $\pm$ 2.7*			Bedoukian Research Inc.
	Coriander Egyptian oil	8	24.2 $\pm$ 4.8*			New Directions Aromatic
	Control	8	12.0 $\pm$ 1.3			
	Arena	8	10.0 $\pm$ 3.9			
II	L-carvone	8	27.5 $\pm$ 3.5*	6.90	0.0001	Bedoukian Research Inc.
	Spearmint oil	8	25.0 $\pm$ 2.2*			Bedoukian Research Inc.
	Styralol	8	16.4 $\pm$ 2.4			Bedoukian Research Inc.
	Control	8	14.6 $\pm$ 1.1			
	Arena	8	16.4 $\pm$ 2.2			
III	Nonanal	8	27.7 $\pm$ 3.2*	4.84	0.002	Sigma-Aldrich Co.
	Benzyl alcohol	8	25.1 $\pm$ 3.4*			Sigma-Aldrich Co.
	6-Methyl-5-Hepten-2-one	8	20.9 $\pm$ 2.5			Sigma-Aldrich Co.
	Control	8	15.1 $\pm$ 1.8			
	Arena	8	11.2 $\pm$ 2.6			
IV	Insect Biting Lure	4	16.8 $\pm$ 1.6	0.57	0.63	Bedoukian Research Inc.
	R-Octenol + NH <sub>3</sub> HCO <sub>3</sub>	4	15.3 $\pm$ 3.3			Bedoukian Research Inc.
	Z-Geranyl Acetone	4	13.0 $\pm$ 2.4			Bedoukian Research Inc.
	Control	4	12.0 $\pm$ 3.7			
	Arena	4	42.7 $\pm$ 3.6			

\* Indicates significantly different from the unbaited control within each group ( $P < 0.05$ ).

was evaluated using the same method as that in Section 2.6. Each of the four traps in each arena was baited with one of these chemicals. Four arenas were used to obtain four replicates.

**2.9. Attractiveness of a Chemical Lure Mixture to Bed Bugs.** Nonanal, 1-octen-3-ol, spearmint oil, and coriander Egyptian oil were confirmed with significant attraction to bed bugs from Section 2.7. We examined the attractiveness of a mixture of these four chemical lures. Ten microliter of each chemical was dispensed onto cotton within a 0.7 mL microcentrifuge tube. The experimental setup was similar to Section 2.7 (Figure 1(b)). Each plastic tray arena had two traps: one trap was used as an unbaited control and the other trap received the chemical lure mixture. Four tray arenas were used. The experiment was repeated the next day. The baited and unbaited trap positions in each arena were switched on the second day. Other procedures were the same as those in Section 2.3.

The attractiveness of the four-chemical lure mixture was also compared with each individual lure component. A 40  $\mu$ L of individual chemical lure was dispensed on cotton within a 0.7 mL microcentrifuge tube. Two traps were placed at opposite corners. One trap received one of the four chemicals and the other trap received the four-chemical lure mixture. Four arenas were used. On each day, a different chemical was tested in each arena. The experiment was repeated four times over four consecutive days following a Latin square design. Other procedures were the same as those in Section 2.3.

## 2.10. Attractiveness of a Chemical Lure Mixture When CO<sub>2</sub> and Heat Are Present

**2.10.1. Comparison between CO<sub>2</sub> Alone and CO<sub>2</sub> + Chemical Lure + Heat.** Two door arenas were baited with CO<sub>2</sub> (200 mL/min) and two arenas were baited with a combination of CO<sub>2</sub> (200 mL/min), heat (4 mini hand warmers), and the chemical lure mixture as discussed in Section 2.9 (Figure 1(c)). The experiment was repeated four times over four consecutive days to obtain 8 replicates. The baited and unbaited trap positions in each arena were switched after two days.

**2.10.2. Comparison between CO<sub>2</sub> Alone and CO<sub>2</sub> + Chemical Lure.** Two door arenas were baited with CO<sub>2</sub> (200 mL/min) and two arenas were baited with a combination of CO<sub>2</sub> (200 mL/min) and the chemical lure mixture. The experiment was repeated three times over three consecutive days to obtain 6 replicates. The baited and unbaited trap positions in each arena were switched after the second day. The experimental procedures were the same as those in Section 2.3.

**2.11. Statistical Analyses.** Bed bug distribution among traps in each arena was summarized as percentage of bed bugs in traps and percentage of bugs that remained in the arena. Generalized mixed linear models (PROC GLIMMIX) were used to analyze the count data [21]. The model accommodates random effects (cohort), repeated measures, and overdispersion. In all experiments, only those bed bugs

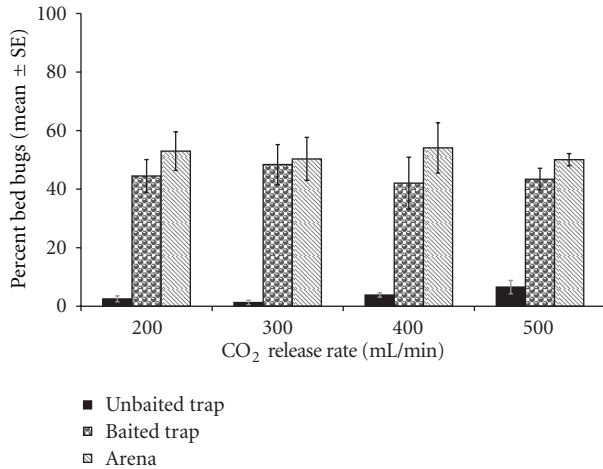


FIGURE 2: Effect of CO<sub>2</sub> release rate on bed bug trap efficacy.

that appeared in the traps were analyzed. Those bugs that remained in the arenas at the end of the experiments were weak, inactive, or behaviorally different from those actively seeking for a host. Previous observations indicate that the presence of bed bugs in a trap had no significant effect on the probability of trapping additional bed bugs. Therefore, the bed bugs in the traps were considered independent events and were not related to gregarious behavior. The data for Sections 2.10.1 and 2.10.2 were pooled for analyzing differences among treatments.

### 3. Results

The different CO<sub>2</sub> release rates had no significant effect on trap catches ( $F = 2.23$ ,  $df = 3$ ,  $P = 0.08$ ) (Figure 2). In each test arena, the probability (mean  $\pm$  95% confidence interval) of bed bugs being caught in a trap baited with 200, 300, 400, and 500 mL/min CO<sub>2</sub> was  $94.6 \pm 2.6$ ,  $97.4 \pm 1.8$ ,  $91.7 \pm 3.2$ , and  $85.9 \pm 4.1\%$ , respectively. Heat (two or four mini hand warmers) significantly increased trap catches ( $P < 0.05$ ) although there were no significant differences between the two heat sources ( $F = 0.08$ ,  $df = 1$ ,  $P = 0.77$ ) (Figure 3). The probability of bed bugs being caught in traps baited with two and four hand warmers was  $64.7 \pm 4.3$  and  $66.4 \pm 3.9\%$ , respectively. There were no significant differences among pitfall traps baited with CO<sub>2</sub> alone or in combination with 2, 3, or 4 hand warmers in door arenas (Figure 4) ( $F = 0.61$ ,  $df = 3$ ,  $P = 0.60$ ). The probability of bed bugs being caught in traps baited with 200 mL/min alone and in combination with 2, 3, and 4 hand warmers was  $93.2 \pm 2.6$ ,  $95.8 \pm 2.0$ ,  $92.2 \pm 2.8$ , and  $91.0 \pm 2.8\%$ , respectively.

Out of the twelve bed bug attractants evaluated in four-choice bioassays, nonanal, 1-octen-3-ol, spearmint oil, coriander Egyptian oil, L-lactic acid, L-carvone, and benzyl alcohol baited traps caught a significantly higher number of bugs than their corresponding controls ( $P < 0.05$ ) (Table 1). In two-choice bioassays, nonanal, spearmint oil, 1-octen-3-ol, and coriander Egyptian oil baited traps caught significantly more bugs than L-lactic acid and L-carvone baited

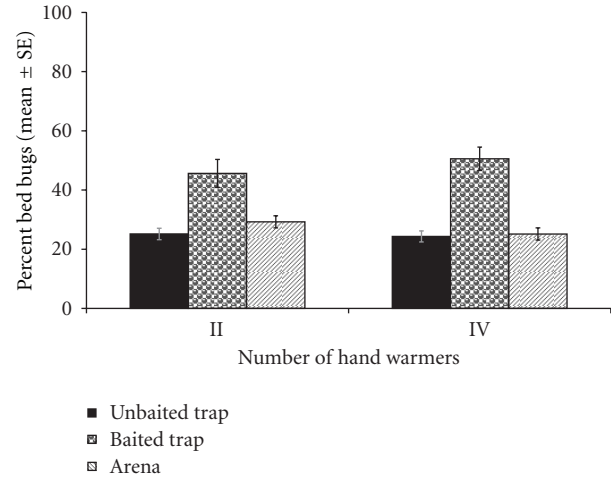


FIGURE 3: Effect of heat on bed bug trap efficacy.

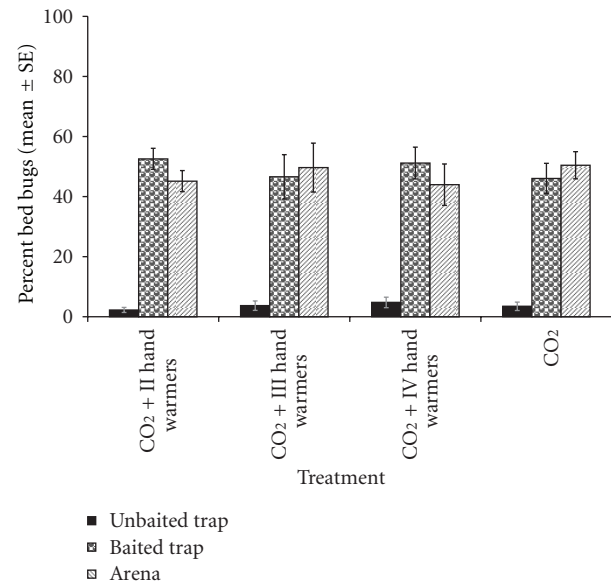


FIGURE 4: Effect of heat on bed bug trap efficacy when CO<sub>2</sub> is present.

traps ( $F = 10.02$ ,  $df = 5$ ,  $P = 0.0001$ ) (Figure 5). Nonanal, spearmint oil, 1-octen-3-ol, and coriander Egyptian oil were not significantly different from each other ( $P > 0.05$ ). The probability of bed bugs caught in traps baited with nonanal, spearmint oil, 1-octen-3-ol, coriander Egyptian oil, L-lactic acid, and L-carvone was  $75.1 \pm 3.3$ ,  $73.9 \pm 3.0$ ,  $69.0 \pm 3.7$ ,  $67.3 \pm 4.0$ ,  $55.2 \pm 3.8$ , and  $51.9 \pm 4.3\%$ , respectively. Further analysis in four-choice experiments showed that pitfall traps baited with nonanal captured a significantly higher number of bed bugs than spearmint oil, 1-octen-3-ol, and coriander Egyptian oil ( $F = 6.43$ ,  $df = 3$ ,  $P = 0.0002$ ). In each arena, the probability of bed bugs being trapped in nonanal, coriander Egyptian oil, 1-octen-3-ol, and spearmint oil baited traps was  $41.5 \pm 4.0$ ,  $19.6 \pm 3.0$ ,  $18.3 \pm 4.0$ , and  $20.6 \pm 4.0\%$ , respectively.

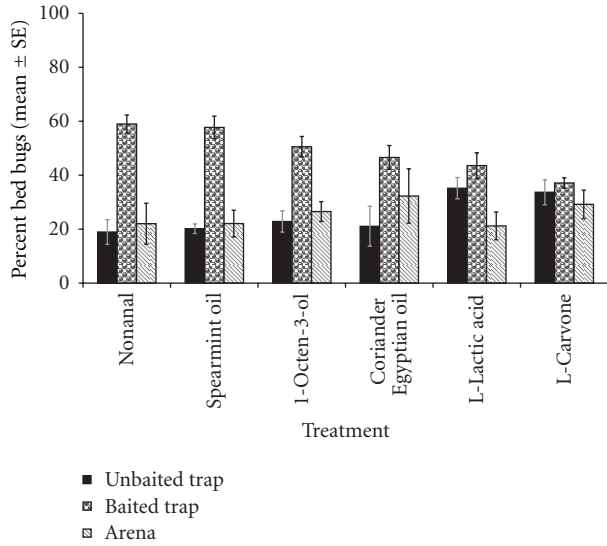


FIGURE 5: Attractiveness of chemical lures to bed bugs in two-choice bioassays.

The traps baited with a chemical lure mixture comprising nonanal, spearmint oil, 1-octen-3-ol, and coriander Egyptian oil captured significantly higher numbers of bed bugs than the unbaited control traps ( $P < 0.05$ ). The probability of bed bugs trapped in chemical lure mixture baited traps was  $71.0 \pm 2.8\%$ . These chemical lure mixture baited traps were significantly more attractive to bed bugs than any of the four individual lure components ( $P < 0.05$ ) (Figure 6). The probability of bed bugs trapped in chemical lure mixture baited traps when compared with nonanal, coriander Egyptian oil, 1-octen-3-ol, or spearmint oil baited traps was  $66.9 \pm 3.6$ ,  $70.4 \pm 3.5$ ,  $71.1 \pm 3.6$ , and  $72.6 \pm 3.4\%$ , respectively. Traps with a combination of either chemical lure mixture + CO<sub>2</sub>, or chemical lure mixture + CO<sub>2</sub> + heat captured significantly more bed bugs when compared to the traps baited with CO<sub>2</sub> only ( $F = 24.81$ ,  $df = 2$ ,  $P = 0.0001$ ). However, bed bug counts in traps baited with chemical lure mixture + CO<sub>2</sub> were not significantly different than those in traps baited with chemical lure mixture + CO<sub>2</sub> + heat ( $P > 0.05$ ). The probability of bed bugs being caught in traps baited with CO<sub>2</sub>, chemical lure mixture + CO<sub>2</sub>, and chemical lure mixture + CO<sub>2</sub> + heat was  $71.7 \pm 1.9$ ,  $87.5 \pm 2.0$ , and  $88.8 \pm 1.7\%$ , respectively (Figure 7).

#### 4. Discussion

Our experiments demonstrated the attractiveness of four chemical lures to bed bugs: nonanal, 1-octen-3-ol, spearmint oil, and coriander Egyptian oil. Among these, nonanal was the most attractive chemical lure. Nonanal has been reported to play a major role in the chemical ecology of triatomine bugs [22], *Aedes aegypti* L. [23], and *Anopheles gambiae* [24]. Nonanal was also the major compound found in odorant profiles of humans, chicken, and pigeon and elicited strong response in antenna of southern house mosquito, *Culex pipiens quinquefasciatus* Say [25]. Traps baited with

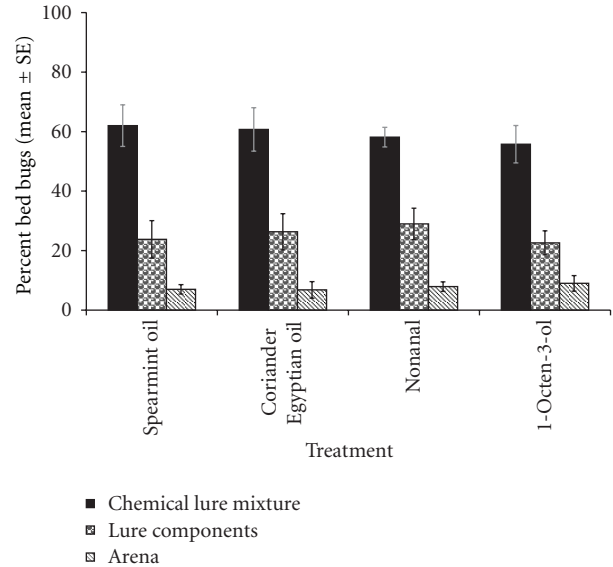


FIGURE 6: Attractiveness of a lure mixture compared with single lures.

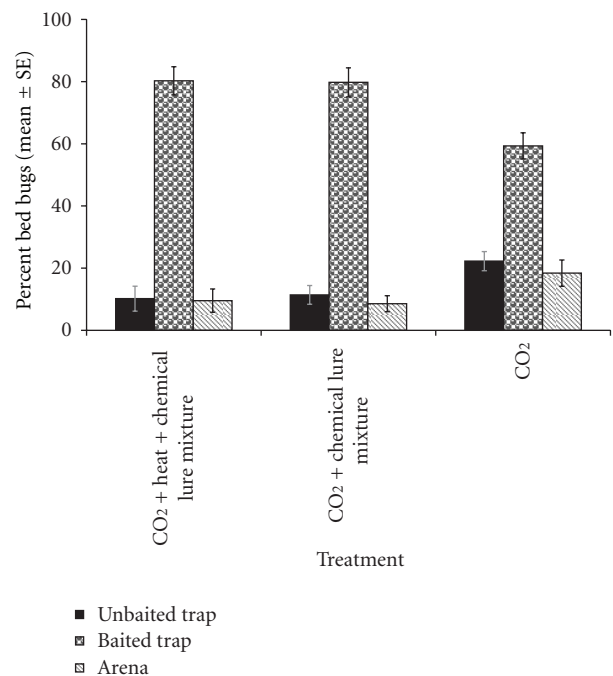


FIGURE 7: Effect of chemical lure mixture and heat + lure mixture when CO<sub>2</sub> is present.

nonanal and CO<sub>2</sub> caught higher number of southern house mosquitoes than traps baited with CO<sub>2</sub> alone [25]. 1-Octen-3-ol has been reported to attract different blood sucking insects including bed bugs [11, 12], *Triatoma infestans* Klug [26], *Glossina* spp. [27], and *Aedes* and *Culex* spp. mosquitoes [28, 29]. Spearmint oil and coriander Egyptian oil are plant derived. L-carvone is the major component (51%) present in spearmint oil [30]. However, L-carvone did not significantly increase trap catch in two-choice bioassays. Its enantiomer,

D-carvone, has been patented as an attractant for Culicidae mosquitoes [31]. Spearmint oil and carvone (L and D enantiomers) were found very attractive to both nymphs and adults of spot clothing wax cicada, *Lycorma delicatula* White [32]. Coriander Egyptian oil has the aroma similar to odors emitted by bed bugs [33].

CO<sub>2</sub> was very attractive to bed bugs regardless of the CO<sub>2</sub> release rates being used when tested in door arenas, indicating that 200 mL/min rate is sufficient for attracting bed bugs in a room that is 2 m in length. Marx [13] and Anderson et al. [11] reported that bed bugs can locate a host that is 150 cm and 86 cm away. The 200 mL/min rate seems to have exceeded the bed bug response threshold and any higher concentrations above that were not helpful in enhancing their responses in door arenas. Measuring the CO<sub>2</sub> gradient at various locations of the arenas might be helpful to establish the relationship between CO<sub>2</sub> release rate and bed bug responses. Under field conditions where a typical room is much larger, the minimum effective CO<sub>2</sub> release rate might be larger. Moreover, bed bug hunger levels, air current, and presence of a human host will affect the minimum effective CO<sub>2</sub> rate.

Adding a mixture of four attractants (nonanal, 1-octen-3-ol, spearmint oil, and coriander Egyptian oil) increased bed bug trap catches when CO<sub>2</sub> was present, indicating the additive effect of chemical lures and CO<sub>2</sub> on bed bug host searching behavior. Similarly, Allan et al. [7] found greater attraction in *Culex* spp. by the combined use of feathers and CO<sub>2</sub> than by using each component alone. Mixture of 1-octen-3-ol with CO<sub>2</sub> was reported to be more attractive than CO<sub>2</sub> alone in *Culex salinarius* [34, 35]. Tropical bont ticks, *Amblyomma variegatum* F., were found to be more attracted to pheromone + CO<sub>2</sub> than CO<sub>2</sub> alone [36]. Host seeking in *A. variegatum* involves activation and a nondirectional searching activity by CO<sub>2</sub> and a directional movement to pheromone and to other host emanating odors [36]. Hematophagous hemipteran, *Triatoma infestans* Klug, which is closely related to *C. lectularius*, also uses a combination of host cues to locate a host. CO<sub>2</sub> served as a long range cue in its nonoriented searching behavior and when a bug arrives in close proximity of its host, then radiant heat and chemical odors from the host oriented it to the exact host location [37]. It is possible that bed bugs host searching behavior follows a similar sequence to that of *T. infestans* or *A. variegatum*.

The presence of either two or four hand warmers (or a 0.8–1.6°C difference in temperature between the lip of the trap and the ambient air) attracted bed bugs from a distance of 25 cm. The role of heat became insignificant when used in combination with CO<sub>2</sub> in wooden door arenas, indicating adding heat when a gradient of CO<sub>2</sub> concentration was present in the environment was not helpful in increasing trap catches. In contrast, the role of chemical lure mixture was significant even when CO<sub>2</sub> was present.

The arena substrates were never cleaned or changed during the study period. They could retain natural attractant/chemical cues, which also persist in natural infestations. We wanted to mimic field conditions and determine if the traps can attract the bugs that were already acclimated to the

arenas with feces and their associated pheromones present. Results from such experimental conditions would more likely correspond well to those obtained under field conditions.

Wang et al. [38] showed the effectiveness of pitfall traps baited with CO<sub>2</sub> alone for detecting very low level bed bug populations. But none of the bed bug monitors provide 100% assurance of the presence/absence of bed bugs in field environments. Results from this study suggest adding an inexpensive chemical lure to a trap may significantly improve the trap efficacy and provide more accurate monitoring of bed bug infestations. Wang et al. [38] suggested that an effective monitor can be used in unoccupied infested rooms to trap the hungry bed bugs and for reducing the probability of bed bugs dispersing into adjacent uninfested rooms. An effective monitoring/trapping system for bed bugs could also be combined with insecticides to kill bed bugs that are attracted to lures or baited traps.

It is noteworthy to mention that the bed bug strain, hunger level, arena size, and test room conditions had significant impacts on test results in our preliminary experiments. Even within a test arena, there could be a location effect. In a ventilated room, bed bugs that are downwind of the baited trap are more likely to be exposed to the plume of CO<sub>2</sub>, chemical lure, or heat. When testing the effect of chemical lures or heat alone, we used small plastic arenas and a room with still air. Using a door-sized arena or in a ventilated room could not detect the attractiveness of chemical lures or heat. When testing CO<sub>2</sub> or combination of CO<sub>2</sub> and heat and/or chemical lure, we used door arenas in ventilated rooms, which mimic the field conditions. Field conditions are usually much more complex than laboratory environments. The presence of a human host, clutter, furniture, and various odors from food and household cleaning agents could significantly affect the performance of a bed bug monitor. Further research is needed to optimize the chemical lure release rate and CO<sub>2</sub> release rate and to evaluate the effectiveness of baited monitors under various field conditions.

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